CERTAIN LIGHT INDUCED CHANGES WHICH CONTROL DRINKING IN THE HAMSTER, MESOCRICETUS AURATUS

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ABSTRACT

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CERTAIN LIGHT INDUCED CHANGES WHICH CONTROL DRINKING IN THE HAMSTER, <u>MESOCRICETUS</u> <u>AURATUS</u>

By

Nicholas S. Van Dyke

Studies were undertaken to determine the effect which different lighting regimes had on the water intake of Mesocricetus auratus. The hamster's water drinking activity recorded with a capacitance-type activity monitor were subjected to spectral analysis and interpeak interval analysis to detect any rhythms which occurred. Total activity data were subjected to the same analysis. In all the test periods, except periods 5 and 6, a circadian rhythm was observed for both water drinking and total activity. However. periods 5 and 6 showed 12 hour peaks. The interpeak interval analysis showed significant 3 hour peaks for both water drinking $(3.00 \pm 0.06 \text{ hours})$ and total activity $(3.05 \pm 0.06 \text{ hours})$ 0.06 hours). The conclusion was drawn that these 3 hour peaks must be superimposed on the circadian rhythm. Also, the 3 hour period in water drinking may indicate a coupling with the system controlling the frequency of urination (Richter, 1965).

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The analysis of water consumption data and interpeak interval data showed that the water intake of the hamster appears to be controlled by at least 2 distinct neural mechanisms, one controlling the frequency of drinking, possibly localized in the suprachiasmatic nuclei (Stetson and Whitmyre, 1976), and one controlling the amount of water consumed, probably in the hypothalamus. The data also suggest that the regulation of drinking has reference points adjusted to the relative surface area, to food intake and probably to changes in body composition.

The data from this thesis demonstrate that the water ingestion control system has a number of subsystems each controlling a different aspect of drinking behavior since the separate end points can be dissociated by manipulation of the environmental photoperiod. The data also show that the circadian periodicity of total activity is controlled more closely than circadian water drinking. This demonstration also depends on the classical physiological testing criterion, namely, that subsystems which may be dissociated (uncoupled) by external conditions (in this case by photoperiods) must have different control mechanisms. Significant linear regression revealed different initial and final activity data in a number of cases. Although a true aging effect can not be ruled out, neither can the cumulative effects of isolation, nor the cumulated results of the successive treatments employed in this study.

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A THESIS

Submitted to Michigan State University in partial fulfillment of the requirements for the degree of

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Dedicated to my parents, Alvin and Sylvia Van Dyke, whose moral and financial support made this research possible.

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TABLE OF CONTENTS

	Page
INTRODUCTION	1
REVIEW OF THE LITERATURE	
Body Water Compartments and Fluxes	6
Studies in Water Intake and Causes of Thirst	8
Normal Drinking	13
Basal Metabolic Rate Studies in Relation	1 5
Fortrainment Studiog	15
	10
METHODS	
Experimental Rationale	19
Experimental Set-up	19
Measurements	22
Statistical Considerations	23
FINDINGS	
1101103	
I. PRIMARY DATA RESULTS	
Effect of Photoperiod Alterations	25
On Mean Water Intake	25
Water Intake	28
Water Intake	40
Chi-square Analysis of the Interpeak	
Intervals of water Drinking Benavior .	50
II. METABOLIC EXTRAPOLATIONS OF PHYSIOLOGICAL SIGNIFICANCE (Estimated from Simple	
Calculations Based on Empiric Equations)	
Comparison of Food Consumption and Weight Gain	53
Food Consumption	57

III.	SPECTRAL ANALYSIS OF HAMSTER MOTOR AND WATER DRINKING ACTIVITY	Page
	Total Activity	. 60 . 62
DISCUSS	ION	. 65
SUMMARY	AND CONCLUSIONS	. 77
LITERATU	JRE CITED	. 79
APPENDIC	CES	
Α.	Calculation of Total Available Water	. 81
Β.	Sample Calculation of the Standard Metabolism for the Hamster	. 82
C.	Sample Data to Determine How Interpeak Intervals May be Determined	. 83
D.	Frequencies Interpeak Intervals, per 10-day Experimental Period	. 85
Ε.	Sample Chi-square Analysis	. 87
F.	HP-65 Linear Regression Program	. 89
G.	Results of Chi-square Analysis	. 92
Н.	Spectral Analysis Values for the Amplitude of the 24 Hour Peak Found in Total Activity and Water Drinking Activity	. 95
I.	Phase Angles and Crest Time for the Hour Peaks Previously Shown in Appendix H	. 96

LIST OF TABLES

Tab1	le
------	----

Page

1.	Provio	des the results of the calculations	
	31001	Water consumption (g/day)	
	Ъ.	Total water available (g/day)	
	с.	Mean body weight (g)	
	d.	Food consumption (g/day)	
	foi	r each of the 12 photoperiods	27
2.	Compar	rison between different photoperiods	
	and th	ne following metabolic, indicators	
	a.	Mean body weight (kg ^{3/4})	
	D.	Water consumption (g/kg ^{3/4} /day)	
	с.	Total water available (g/kg ³ /4/day)	70
	u.	$(g/kg^{3/4}/day) \cdots \cdots$	30
3.	The ef	fect of photoperiod on additional	
	comput	ed indicators	
	а.	Total activity (C _O ; mean counts/hour)	
	b.	Water drinking activity (C _O ;	
	_	mean counts/hour)	
	с.	Total activity minus water drinking	
	A	activity The mg of water per lick a second second	33
	u.	The mg of water per fick	55
4.	The ef	fects of different photoperiods on	
	derive	d indicators	
	а.	Energy from the food consumed	
	1	(kcal/day)	
	D.	Mean daily standard metabolism	
	c	(KCal/day) Kcal consumed per day minus the	
	ι.	kcal needed for basal metabolism	
		(kcal/day)	
	d.	The mean weight gain per day (grams/day)	
	e.	The food energy remaining for activity	
		after the standard metabolism and	
		weight changes are taken into	5 4
		account (kcal/day) •••••••••	50

Table

5.	A compa for the	arison of the different indicators e system controlling water balance	
	а.	Water consumption $(g/kg^{3/4}/day)$	
	Ъ.	Milligrams of water per lick	
	с.	Water drinking activity mean	
		interpeak intervals (hours)	
	d.	C_{24}/C_0 ratio for water drinking	
		²⁴ activity	
	е.	Phase angle for water drinking	
		activity (degrees)	
	f.	Period length for water drinking	
		activity (hours)	
	for	each of the 12 photoperiods	67
6.	A compa	arison of different indicators relating	
•••	to tota	al activity	
	a 10 10 10	Total water available $(\sigma/k\sigma^3/4/dav)$	
	ь. h	Total activity mean interneak	
	υ.	intervals (hours)	
	c	C_{α}/C_{α} ratio for total activity	
	с. а	Dease angle for total activity (degrees)	
	a.	Priod longth for total activity (degrees)	
	e.	Period length for total activity (nours)	60
	for	each of the 12 photoperiods $\cdots \cdots \cdots$	09

LIST OF FIGURES

Fig	ure		F	Page
1.	Relation of total activity (C _O ; mean counts/hour) to water consumption (grams/day)	•	•	35
2.	Bar graph representing the total activity (C ₀ ; mean counts/hour) divided by the water consumption (grams/day) during each of the 12 different periods			37
3.	Bar graph representing the total activity (C _O ; mean counts/hour) during each of the 12 different periods	•	•	37
4.	Relation of total activity (2000-0400 hours) to water drinking activity (2000-0400 hours)	•	•	39
5.	Relation of total activity (2000-0400 hours) to water drinking activity (1900-0500 hours)		•	42
6.	Relation of food consumption (grams/day) to water consumption (grams/day)	, •	•	45
7.	Bar graph representing the food consumption (grams/day) divided by the water consumption (grams/day) during each of the 12 different periods	• •	•	47
8.	Bar graph representing the food consumption (grams/day) during each of the 12 different periods.	• •	• •	47
9.	Relation of food consumption (grams/day) to water drinking activity (C ₀ ; mean counts/hour)	• •	• •	49
10.	Relation of food consumption (grams/day) to total activity (C _O ; mean counts/hour) .	• •	, .	59

INTRODUCTION

In the experimental investigation of thirst it is usual to regard the amount of water an animal drinks as a quantitative measure of the thirst that animal experiences. Other behavioral measurements such as the rate of drinking, and the "point" at which an animal will consume a normally adversive solution can also be used as a measure of thirst. However, it is the amount of water ingested that can be most directly related to the need and therefore presumably to the intensity of the original sensation.

The mechanisms involved in controlling the drinking responses of an animal are genetically determined, innate, but tend to mature as the animal matures. Adolf (1947) found that drinking in response to a thirst stimulus is deficient at first. Experiments have shown that the 19 day old rat drinks at a slower rate after intragastric hypertonic NaCl than does the older animal. Stricker (1966) found that drinking after administration of hypertonic NaCl is absent or much reduced before the nineteenth day, but that the responses reach the adult levels by about the twenty-first day. It is worth noting that drinking response to both extracellular and cellular stimuli appear at the same stage in the animal's development. This is probably related to maturation of the hypothalamus, since other

hypothalamic functions in the rat also reach their full development at about this time.

There are two major fluid compartments in the body, the cellular space and the extracellular space. The theory that drinking is caused by either a relative or an absolute lack of water, requires receptors monitoring each of these compartments. Each compartment has its own intake controlling mechanism which is independent of the other, but the two mechanisms have an additive effect on drinking when activated together.

The stimulus for cellular control is cellular shrinkage (hypovolia), with some receptors at least in the hypothalamus. A thirst for water results, and water is the appropriate restoring fluid. The response of the control system to stimulation makes it reasonable to suppose that cellular receptor volume fluctuates between an upper satiety level and a lower ingestion threshold level. However, because there is much anticipatory drinking, the threshold for cellular thirst is seldom reached.

Wolf (1950) calculated a thirst threshold in the dog after infusing hypertonic NaCl intravenously and measuring the amount of saline needed to induce drinking. He calculated that the drinking threshold in the dog is reached when the cells have shrunk by only 1 or 2% of their initial volume. This result is similar to the value of 1 or 2% increase in osmolality found by Verney (1947) to stimulate the ADHreleasing osmoreceptors. Wolf proposed that the same or

si ne 1.0 th ri iċ ne T(ŧ similar osmoreceptors constitute the primary afferent component of the thirst reflex. Thus Wolf suggests that hypovolia receptors and osmoreceptors may be the same. Hence there is a general tendency to refer to "osmoreceptor theories of the regulation of drinking". Since the morphological identification of the cells responsible for such different neurophysiological functions is still obscure, the concept remains conjectural.

Hypovolemia (in contrast to hypovolia) appears to be the stimulus for extracellular thirst, with the receptors located in some low pressure part of circulation. There is an initial thirst for water, but the water intake response is generally not sufficient by itself to completely repair the plasma deficit. The quantities that may be ingested are limited by increasing hypotonicity which has been postulated to inhibit further drinking (Stricker, 1969). However, the development of a delayed preference for a saline solution ensures an intake of a fluid that is homeostatically more appropriate, and under these circumstances the plasma volume is restored to normal. In extracellular thirst the renin-angiotensin system appears to participate. Only 5ng of angiotensin II injected into the anterior hypothalamic region causes a rat in normal water balance to drink water. The involvement of the renin-angiotensin system in extracellular thirst is extremely interesting, since it provides a means for linking two important extracellular controls,

the ingestion of water and the retention of sodium by the kidney through the intermediary of aldosterone.

Both hypovolia and hypovolemia are causes of "true" thirst. However, an animal usually does not drink because it is "truly" thirsty. Need for water is not a motive in the psychological sense. In rats there appears to be a circadian rhythm of drinking modulated by that nature of the diet. Fitzsimmons and LeMagnen (1969) found that feeding and drinking are closely associated, and that between 80 and 85% of all food and water ingested is taken during hours of darkness.

At least 70% of the total intake of water is drunk just before, during and immediately after meals, and there is a highly significant positive correlation between the amount of water associated with a particular meal and the size of that meal (Fitzsimmons and LeMagnen, 1969). There are a number of possible explanations for this close relationship between feeding and drinking.

- Eating and drinking may each be linked to the 24hour activity rhythm.
- 2. The taste of food, its smell or its presence in the mouth or stomach might cause an anticipatory intake of water in amounts appropriate to the future needs of the animal. Nicolaidis (1968) found it necessary to postulate buccal receptors responsive to changes in osmolality in the mouth afferent to hypothalamic osmoreceptors. These

might well be the receptors concerned in anticipatory drinking.

3. Ingestion of food eventually causes a temporary deficit in total circulating extracellular fluid volume through increased secretion of isotonic digestive juices. This deficit might result in some sort of a thirst stimulus.

While the nature of the diet, through oropharyngeal and possibly gastric fullness mechanisms, influences the quantity of water ingested with meals, there is also an underlying rhythm of drinking which accounts for the nocturnal predominance of drinking.

This thesis deals primarily with photo-periods and their effect on water drinking activity in the hamster.

REVIEW OF THE LITERATURE

Body Water Compartments and Fluxes

As suggested by Reeve and Kulhanek (1967), body water can be represented as being contained in three compartments. As illustrated below, these compartment are gut water (W_G) , extracellular water (W_E) and the cellular water (W_C) . Each of the three compartments



diagramed has its own inputs and outputs. Ignoring feed water loss gut by absorption, K_1W_G . Extracellular water, W_E , has inputs, K_1W_G , and cellular water influx into W_E , K_3W_C . Outputs are two kinds of water vapor loss, here collectively indicated as L, urinary excretion, u, and water transfer to the cells, K_2W_E . Cellular water, W_C , has input, K_2W_E , and output, K_3W_C .

hence,
$$dW_G/dt = W_G = d - K_1 W_G$$

 $dW_E/dt = W_E = K_1 W_G + K_3 W_C - K_2 W_E - u - L$
 $dW_C/dt = W_C = K_2 W_E - K_3 W_C$

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It should be noticed that these equations are flux equations, across surfaces which are unspecified here and by a number of mechanisms. The form of the relationships indicated by the equations is not useful for modeling on the computer, since the physiologically interesting relationships as expressed in this model are essentially static. However, we know that water balance is not only a static phenomena and that very large degrees of dehydration as well as the upper extreme, water intoxication, are compatable with life. Secondly, body water kinetics, particularly the transients, are incompletely investigated, although steady state relationships are commonly utilized in clinical medicine. Further, the utility of this representation is dependent upon additional assumptions, primarily about (a) the order of the transfer constants and the fact that (b) transient vs. steady state relationships are not explicit. Thus mathematical integrations are required which are not yet well defined. Finally, this model is suggestive of additional elements which might require a more sophisticated analysis of subsystem behavior. However, if L is assumed constant and the low rates of water vaporized through lungs and skin as well as the fecal water loss are all considered to be neglegible, then it can be seen that there are only two quantitatively important and variable rates of exchange of water between the body and its environment, d and u. These are the major controlled variables of the body water system which maintains water balance between input by drinking and

and dis dif mai pe fì tl i Ņ and output by urinary water excretion. The emphasis of this discussion will be on water drinking and the effects which different photo-periods have on it. Since water balance remains within physiological limits, the effects of the photoperiods on drinking are expected to also influence urination.

Studies in Water Intake and Causes of Thirst

It was suggested very early that the loss of water from the tissues so altered the properties of the cells that they collectively initiated the sensation of thirst.

Holmes and Gregerson (1950) found that when dogs were injected intravenously with hypertonic solutions of NaCl, Na_2SO_4 , sucrose, or sorbitol, all substances which cause fluid to leave the cells, the dogs drank more water than when they were given hypertonic urea, isomannide, or glucose, substances which penetrate cells easily. A logical conclusion that might be drawn from the above results is that cellular dehydration rather than an increase in cellular osmotic pressure is the true stimulus for true thirst. This conclusion is based on the assumption that cells act as perfect osmometers.

The threshold of thirst in man and dog was calculated by Wolf (1950) to be reached when the cells had shrunk by 1 or 2% of their initial size, much the same figure that Verney had found for the osmotic threshold of the ADH receptors. Wolf calculated the threshold of thirst by infusing hypertonic NaCL intravenously and measuring the amount of

sa] th ca as se th ve ec e) p. g 0 t С saline needed to arouse thirst, or in the case of the dog, the amount needed to start the dog drinking. In making his calculations, Wolf assumed that the cells of the body behave as perfect osmometers, and that it is cellular shrinkage itself which gives rise to thirst. He suggested that the thirst "osmoreceptors" may be situated in the hypothalamus.

Consider what happens when a solute is infused intravenously. A hypertonic solution of a solute that is excluded from the cells initially increases the osmolality of the extracellular fluid. However, even as the rise is taking place, water is withdrawn from the cells so that the osmotic gradient across the cell membranes, tends to disappear. Its own final concentration and the effect it has on the concentration of other constituents, however, depend on the nature of the solute and on the fluid shifts that it produces in the body. In this way the increase in osmolality is distributed throughout the fluids of the body, though the solute causing the rise is confined to the extracellular space. The end result is a transient overall increase in cellular as well as extracellular osmolality with a decrease in cell size and an increase in extracellular fluid space.

Wolf (1950) defined thresholds of thirst and drinking caused by the slow infusions of hypertonic saline in terms of the 1% decrease in fractional cellular water content, T (note change in symbols), that is believed to be present at the moment of onset of thirst and the initiation of drinking.

The percentage decrease in cellular water at threshold,

100 T =
$$\frac{(Vi - Vi')}{Vi}$$

Vi = initial cell volume
Vi' = final cell volume

averaged 1.23 ± 0.48% in man and 2.15 ± 0.64% in the dog. Losses of less than this amount will not cause "true thirst", though of course drinking may occur for quite different reasons. Once the "threshold" has been reached, however, drinking starts and continues until enough water has been ingested to restore the body fluid to a more "normal" level whether or not transient satiety mechanisms intervene.

Since this threshold is similar to the value of 1 - 2% increase in osmolality found by Verney (1947) to stimulate the ADH releasing osmoreceptors, Wolf proposed that the same or similar osmoreceptors lie on the afferent side of a thirst reflex. This idea has received partial experimental confirmation by Anderson (1953) who caused immediate polydipsia in goats by injecting 0.1 - 0.2 ml of 2% NaCl into the perifornical region.

It is most improbable that only the cellular compartment is controlled by the thirst mechanism. Maintenance of the extracellular compartment is perhaps more important for the short-term survival of the animal in view of the importance of the circulatory volume. The extracellular volume is less than half the volume of the cellular compartment, yet it is the principal place where exchanges with the

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external environment take place, and it is involved in the very large internal turnover of fluid in the kidneys and gastrointestinal tract.

An outline for a possible mechanism of extracellular thirst is as follows:



The cause of extracellular thirst is more complicated, since there is usually a deficit of Na as well as water. Water alone is not sufficient to restore the extracellular space; the plasma deficit especially is little affected by the amounts of water that are ingested before further drinking is inhibited by osmotic dilution of the body fluids, (Stricker, 1969). Even if larger amounts of water were drunk, the vascular volume would benefit little, since the additional water would be distributed throughout the body fluids. In other words, though there is a rough correspondence between the size of an extracellular deficit and the amount of water ingested, this water may have very little effect on the plasma volume. Yet despite the continuing plasma volume deficit, drinking of water often ceases. A further intake may lead to water intoxication.

The commonest cause of thirst is water deprivation. Though it is not known with certainty how the water deficit produced by deprivation is shared between the cellular and extracellular compartments, it is thought (Dicker, 1949) that initially water is lost from the extracellular space and then from both cellular and extracellular compartments in proportion to their normal size. With more severe depletions, the extracellular fluid is sustained at the expense of the cellular fluid, the so called dehydration reaction (Elkinton and Taffel, 1942). Since both compartments are affected, it is of interest to consider whether cellular and extracellular mechanisms can be additive in their effects on drinking, each contributing to the total thirst experience.

The prediction of additivity is supported by the experimental evidence for a wide variety of thirst stimuli. Oatley (1964, 1967) found that the combination of hemorrhage, an extracellular stimulus of thirst, and hypertonic saline, a cellular stimulus, produced more drinking than either stimulus presented alone. This work was extended (Fitzsimmons and Oatley, 1968), and it was found that deprivation of water, a stimulus which depletes both major fluid compartments, combined with injection of hypertonic saline or with hemorrhage, yielded drinking which was a simple addition of the effects of the two stimuli. In all cases observed, drinking produced by one stimulus simply adds to drinking produced by the other. The absence of interaction

between the components in a combination stimulus provides evidence for the functional independence of the systems mediating cellular and extracellular thirst. These have also been shown to be neurologically independent (Blass, 1968).

Normal Drinking

Appetite for water is not just a mild form of thirst, but is an acquired drive dependent on previous pleasure experiences. Appetite causes the animal to drink for pleasure, there is no underlying need for water, whereas thirst causes drinking to relieve a physiological imbalance.

Continuous recordings of feeding and drinking in a room lit by artificial light controlled by a time switch show that the two phenomena are closely associated (Fitzsimmons and LeMagnen, 1969) and that between 80 and 85% of all food and water ingested is taken during the hours of darkness. However, some feeding and drinking take place at other times, and very often there is a period of accelerated intake of food and water just before the lights are switched off. This suggests that though the rat is depending ultimately on the cycle of light and dark to regulate its activities, it nevertheless has its own 24 hour clock which allows it to anticipate darkness. There is no initial peak of water intake which would be expected were the rat to wake up thirsty. Instead, drinking continues at a fairly steady rate throughout the night.

To test to what extent the normal pattern of drinking is set by fluid needs of the animal, rats were given their expected water requirements by continuous infusion through a soft polyvinyl chloride tube permanently implanted in the stomach.

Four rats infused at a rate of 1 ml per hour for periods of 5 and 7 days and whose sole water supply was provided by infusion maintained normal food intakes and body weights during this period. This amount of water is therefore sufficient for fluid balance. When rats were infused at this rate and were allowed access to drinking water, drinking was only partly suppressed, and the previous pattern of nocturnal predominance persisted. Intragastric infusion of water therefore suppresses drinking rather less than would be expected were need for water the only factor causing drinking.

If an animal normally drinks only when it is thirsty, then continuous infusion of water, particularly at night when most drinking occurs, should inhibit drinking in direct proportion to the amount of water infused. This does not happen. Therefore, need for water, signaled by thirst, is not the cause of normal drinking when food and water are freely available.

Drinking and feeding are closely related in the dog and rat when food and water are freely available. Simultaneous recordings of eating and drinking show there is a very lose temperal relationship between the two activities. At

least 70% of the total intake of water is drunk just before, during, and immediately after meals, and there is furthermore a highly significant positive correlation between the amount of water associated with a particular meal and the size of that meal.

Food-associated drinking seems to be a learned response based on previous experience of the dipsogenic properties of the diet which enables an animal under stable conditions to anticipate its future water requirements by drinking appropriate amounts of water at meal times. The water taken is related to the metabolic needs of the animal; this type of drinking is therefore regulatory. While the nature of the diet determines the quantity of water to be associated with the meals, underlying this intake is a circadian rhythm of drinking which accounts for the nocturnal predominance of drinking.

Basal Metabolic Rate Studies in Relation to Body Size

Fasting animals burn up their body substance and die when they have consumed about one half of their body mass. The body substance is apparently used as a fuel, which can be inferred from the observations that;

 The consumption of body substance during fast increases with increasing thermostatic heat requirements.

- We can express the life-prolonging property of body substance in terms of calories, that is, as the heat of combustion.
- 3. Heating the environment saves the body substance and prolongs life in starvation, however, there is both a lower critical temperature and an upper critical temperature, above which homeothermic control is lost.

The basal metabolic rate is the minimum rate of heat production of a fasting, resting animal at ideal environmental temperature. This rate can be calculated by the relation,

 $B = 70 W^{3/4}$ where, B = basal metabolic rate in kcal per day W = body weight in kg

(Kleiber, 1961)

According to this equation a hamster weighing 80 grams should produce 10.6 kcal of heat daily.

Entrainment Studies

Entrainment of circadian rhythms is the phenomenon whereby a periodically repeated stimulus, such as a light cycle, causes an overt persistent rhythm to become periodic with the same frequency as the entraining cycle. Thus, there is a fixed phase relationship between the entrained rhythm and the entraining cycle. Any time series regardless of waveform, may be analyzed (by means of Fourier's theorem) into an additive assembly of cosines whose parameters (i.e. frequencies, amplitudes and phase angles) can then be determined. In general, the procedure involves converting the data from a function of time to a function of frequency. The spectrum thus obtained will show whatever periodic components (rhythms) are necessary, with their amplitudes and phase angles for each respective frequency, and which sum to the complex functions of time which exist in the original data (the time series).

Norton (1975) found that both constant light and constant darkness caused a general depression of the mean activity levels, however, constant darkness may cause an initial transient increase. This is usually accompanied by a reduction in circadian amplitude thus reducing the C_{24}/C_0 (ratio of the amplitude of the 24 hour peak to the mean value) ratio. There is also a slight shift in frequency which normally accompanies such a modulation.

Stetson and Whitmore (1976) produced evidence which suggests that the suprachiasmatic nuclei may contain a primary oscillator or a coupled oscillator in a two- or multi-oscillator system which regulates a variety of lightsensitive rhythms. A well known visual pathway from the retina to the suprachiasmatic nuclei exists and a number of unrelated rhythms (ranging from locomotor activity to

adrenal corticosterone content) are abolished when the suprachiasmatic nuclei are destroyed. It is unlikely, though not impossible, that a single driving oscillator would be involved in the expression of so many different rhythms. Hence a multi-oscillator concept seems to be suggested.
METHODS

Experimental Rationale

Recording gross motor activity for the hamster using a capacitance activity monitor combines the different "motor subsets" into one printer readout. These different "motor subsets", recorded at the end of each 0.1 hour, add to the total activity readout, each having varying durations and intensities. When combining the different "motor subsets" into one total activity count it must be assumed that the ratio of these different activities remains essentially constant. The total activity count can then be compared to the water drinking activity to determine what effect it has on water consumption. Such an analysis may lead to a better understanding of the mechanisms controlling water drinking in the hamster.

Experimental Set-up

A single young male golden hamster, <u>Mesocricetus aura-</u> <u>tus</u>, was housed under a varying lighting regimen which consisted of Fluorescent light (650 lux) alternating with darkness. In periods one through eleven, food (instant Quaker oats) and water were provided ad libitum and replenished

between periods. Food was not provided during period twelve, however, water was provided ad libitum.

Motor activity and water drinking activity were measured using two capacitance type activity monitors (Stoelting Co., Model #31400, Chicago, IL) equipped with a 6-digit printing counter (Stoelting Co., Model #22408). The movement or drinking of the animal resulted in changes in the capacitance field causing a "count" on the respective counter. Counts were integrated over a six minute interval and recorded hourly. After each print, the counter automatically resets to zero. Thus, 10 days of monitored activity (one period) consisted of 240 recorded data points, one for each hour of clock time. Records were also kept of the average daily food and water intake and weight changes of the hamster.

Recordings were obtained from the hamster maintained in a Habitrail cage (Metaframe Corp., East Patterson, NJ) fitted with an "isolated nesting box" which effectively positioned the animal above the electrical field and eliminated small extraneous counts during sleep. The cage was positioned on an activity monitor and the entire unit enclosed in a chamber covered with a heavy black Visqueen; the water bottle was set up on a separate activity monitor. Thus, recorded data represents "total activity" of the hamster on one printer and the "water drinking activity" on the other printer. Four fluorescent lights (cage light intensity 650 lux) controlled by a time switch, imposed a

24-hour, light-dark, variable photo-period. Cage temp. averaged 29 \pm 2⁰C during the course of the experiment.

A total of twelve, 10-day, periods were obtained before the death of the animal. Changes in the hamster's total activity and food consumption and the effect they had on the water consumption, during these periods, were of particular interest. Hence, linear regression analysis was performed on each of these different areas of interest.

The lighting regimes for the twelve periods reported herein are listed in the following table.

Lighting Regimes

Period	#	Light-dark photoperiod
1		LD _{12:12} (lights on 0600-1800 hours)
2		LD _{16:8} (lights on 0400-2000 hours)
3		LD _{16:8} (lights on 0400-2000 hours)
4		LD _{16:8} (lights on 0400-2000 hours)
5		LDLD _{8:4:8:4} (lights on 0200-1000 hrs and 1400-2200 hrs)
6		LDLD _{8:4:8:4} (lights on 0200-1000 hrs and 1400-2200 hrs)
7		LD _{16:8} (lights on 0400-2000 hours)
8		LD _{16:8} (lights on 0400-2000 hours)
9		LDL _{16:8} (lights on 0400-2000 hours and dim light on 2000-0400 hours)
10		LDL _{16:8} (lights on 0400-2000 hours and dim light on 2000-0400 hours)
11		LD _{16:8} (lights on 0400-2000 hours)
12		LD _{16:8} (no food) (lights on 0400-2000 hours)

Measurements

Measurements of food consumption, water consumption and weight of the hamster were made at the beginning and the end of each 10 day period. The average daily consumption of food and water was calculated from these data by dividing the difference by the number of days between measurements. Also, the hamster's average weight and daily gain or loss for each 10 day period was calculated. The average weight was calculated by dividing the sum of the weights at the beginning and end of each period by two. Since only one measurement was made on these variables no standard errors could be calculated from such data. The water drinking activity and total activity counts for the animal were printed out at six minute intervals and subsequently over an hour for statistical analysis. These counts were the result of the animals movements and drinking which caused a change in the capacitance fields of the monitors. Thus, 10 days of monitored activity consisted of 240 recorded data points for each of the two measurements.

Examples of the calculations of total water intake and basal metabolic rate are given in Appendices A and B, respectively.

Statistical Considerations

Interpeak intervals for the water drinking activity data (Appendix C) were determined for each of the 12 periods. This was done by determining the number of hours between peaks in the water drinking activity. Appendix D shows the results. Since the interpeak interval was not normally distributed a chi-square analysis of the frequencies in the different data cells was done on the interpeak intervals. This chi-square analysis was done to test the homogeneity of the different periods. A sample chi-square analysis is shown in Appendix E.

Linear regression analysis was done on the following relationships.

- Relation of total activity (C₀; mean counts/hour) to water consumption (grams/day).
- Relation of total activity (2000-0400 hours) to water drinking activity (2000-0400 hours).
- Relation of total activity (2000-0400 hours) to water drinking activity (1900-0500 hours).
- Relation of food consumption (grams/day) to water consumption (grams/day).
- Relation of food consumption (grams/day) to water drinking activity (C₀; mean counts/hour).
- 6. Relation of food consumption (grams/day) to total activity (C_0 ; mean counts/hour).

These regressions were computed on an HP-65 hand calculator

to determine the line of best fit to the data points. The coefficient of determination (r^2) , which can be interpreted as the proportion of total variation about the mean \overline{y} explained by the regression, was calculated, as was the correlation coefficient (r) itself. Least squares standard errors were determined for the y-intercept and slope of each of the lines. The standard error of estimate of y on x (S y·x) was also determined for each of the linear regressions. The equations used in the HP-65 calculator programs are shown in Appendix F. The legend for each figure contains the appropriate linear equation with its statistical indicators.

Interpretation of the results is made by considering first, whether the calculated values for the slope and intercept were significantly different from zero. Corraboration of the tests was obtained from the r^2 value.

FINDINGS

I. PRIMARY DATA RESULTS

Effect of Photoperiod Alterations on Mean Water Intake

During each photoperiod the water consumption of the animal was measured by weight and the average daily intake of water calculated. Additional calculations were made to estimate the hamsters total available water. Food water, metabolic water and water from fat loss (See Appendix A) were added to the water consumed to provide an estimate of the total water available to the animal. Table 1 shows the results. During periods 1-11 the average water consumed per day was 7.04 \pm 0.47 grams per day, which is about 76% of the average total water available, 9.21 \pm 0.56 grams per day.

During the first ten day period, a 24-hour light-dark photoperiod (LD_{12:12}; lights on 0600-1800 hours), the animal had an average water consumption and total water intake very close to the average, as was also the case for periods 2, 3, 4, 7, 8 and 11 (LD_{16:8}; lights on 0400-2000 hours). This was expected since these were control periods with a normal (LD_{16:8}; lights on 0400-2000 hours) lighting regime.

Periods 5 and 6 (LDLD_{8:4:8:4}; lights on 0200-1000 and 1400-2200 hours) resulted in probably a slightly increased

Provides the results of the calculations showing, Table 1.

- Water consumption (g/day)
- 2. Total water available (g/day)
- 3. Mean body weight (g)
- 4. Food consumption (g/day)

for each of the 12 photoperiods.

Photoperiod	Period #	Water cons. (g/day)	Tot. Water Avail. (g/day)	Mean Body Weight (g)	Food cons. (g/day)
LD12:12	1	7.02	9.08	56.4	3.41
LD16:8	2	7.23	9.33	68.0	3.47
LD _{16:8}	3	7.23	9.33	68.0	3.47
LD _{16:8}	4	8.19	10.92	79.8	4.52
LDLD8:4:8:4	S	7.83	10.09	80.0	3.75
LDLD8:4:8:4	9	7.83	10.09	80.0	3.75
LD _{16:8}	7	6.52	8.54	79.2	3.38
LD _{16:8}	ø	6.52	8.56	79.2	3.38
LDL _{16:8}	6	6.36	8.33	77.8	3.27
LDL _{16:8}	10	6.36	8.33	77.8	3.27
LD _{16:8}	11	6.36	8.70	77.3	3.88
LD _{16:8} (no food)	12	3.55	5.81	66.6	0.

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) | ka water consumption. Periods 9 and 10 (LDL_{16:8}; lights on 0400-2000 hours and with a dim light on from 2000-0400 hours) resulted in possibly a slight decrease in water consumed. These very slight differences from the general average during periods 5, 6, 9 and 10 probably reflect the changes in body weight or surface area, since correcting the values to body weight to the 3/4 power gives a different picture (Table 2).

Period 12 (LD_{16:8}; lights on 0400-2000 hours) has a water consumption about one-half as large as the average, even though there is a normal lighting regime during this period. The reason for this decreased water consumption can not be related to the lighting regime but rather to the imposed starvation.

From the observations, it is suggested that the regulation of drinking has reference points adjusted to relative surface area, to food intake and probably to changes in body composition. It is also suggested that these physiological variables can be altered both by age of the animal and by exposure to different lighting schedules.

Comparison of Total Activity and Water Intake

Total activity by the hamster was recorded during the 12 photoperiods, as was the water consumption. The average hourly total activity (C_0 ; mean counts/hour) of the hamster was also calculated and compared to the average daily water

- Comparison between different photoperiods and the following metabolic indicators Table 2.
 - 1. Mean body weight (kg^{3/4})
- Water consumption (g/kg^{3/4}/day)
 Total water available (g/kg^{3/4}/day)
 - 4. Food consumption (g/kg^{3/4}/day)

pho	toneriod	Mean body _{3/4})* weight (kg ^{3/4})*	Water cons. g/kg ^{3/4} /day	Tot Wat Avail. g/kg ^{3/4} /day	Food cons. g/kg ^{3/4} /day
1.	LD1,.,1,	0.116	60.7	78.5	29.5
2.	LD, 5 . 0	0.133	54.3	70.0	26.1
	LD	0.133	54.3	70.0	26.1
7	- 10:8	0.150	54.6	72.8	30.1
- u	8:01	0.150	52.1	67.1	24.9
	1 DI D	0.150	52.1	67.1	24.9
o 1	1.1. 1.1.	0.149	43.7	57.3	22.6
• •	8:91 ⁴¹	0.149	43.7	57.3	22.6
8	LU16:8	0.147	43.2	56.6	22.2
С	16:8 ביי	0.147	43.2	56.6	22.2
	LDL16:8	0.147	43.4	59.3	26.1
•	LU16:8	od) 0.131	27.1	44.3	, , ,
2.	LU16:8 (IIU 10	Se he se fet	rimate of rel	ative surface ar	ea.

n ۵ * Sometimes regarded as an estimate of relati

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consumption (Table 1) which was calculated earlier. Table 3 shows the results of these calculations.

A graph (Figure 1) was prepared from these data points and a linear regression analysis was run on the HP-65 (Hewlett-Packard programable calculator) to find the line which best fit the points. As indicated in the legend for Figure 1 the line had a y-intercept of 251.4 \pm 156.2, which is not significantly different from zero. Nor is the slope significantly different from zero, having a value of 0.08 \pm 22.81. Also, there is no significant correlation (r = +0.0011) between the mean daily water consumption and the mean hourly total activity (C₀) of the hamster under the conditions of these experiments. A bar graph further illustrates this point (Figures 2 & 3).

The mean total hourly activity during darkness (2000-0400 hours, when most of the locomotor activity occurred) in periods 2 and 3 was then compared to the water drinking activity during darkness (2000-0400 hours). Linear regression analysis (Figure 4) of these data showed a highly significant correlation of ± 0.90 between the total activity and the water drinking activity during darkness. The yintercept of the best fitting line describing this relationship is -421.0 ± 551.3 which is not significantly different from zero. Because a peak in water drinking activity was very often observed just before the lights went off and just after the lights went on, total activity during the dark time (2000-0400 hours) was also compared to water

- The effect of photoperiod on additional computed indicators Table 3.
- 1. Total activity $(C_0; mean counts/hour)$
- 2. Water drinking activity (C_0 ; mean counts/hour)
- 3. Total activity minus water drinking activity
- 4. The mg of water per lick

Der	.ind # ((fotal Act	ivity ounts/hr)	Water Drin (C _O ;mean co	king Act. unts/hr) 1	Total Activity Water Drinking	minus Act.	ШĞ	/1:	ick
1.	LD. 2.1.2	0 343 . 9	± 30.3	189.8 ±	16.6	154.1 ± 34. ∶	5 1	. 54	+1	0.13
2.	LD, 6 . 8	211.3	± 21.0	84.3 ±	7.1	127.0 ± 22.	2 3	.58	+1	0.30
3.	LD16.8	260.6	± 47.7	85.6 ±	8.1	175.0 ± 48.4	4 3	.52	+1	0.33
4.	LD,6.8	251.2	± 24.8	97.1 ±	7.7	154.1 ± 26. (0 3	.52	+1	0.28
ъ.	LDLD _{8.1.8.4}	270.7	± 27.2	97.8 ±	7.6	172.9 ± 28.	2 3	.34	+1	0.26
. 9	LDLD8.4.8.4	232.4	± 18.6	85.9 ±	6.7	146.5 ± 19.8	8	. 80	+1	0.30
7.	LD16.8	289.4	± 25.6	89.1 ±	6.6	200.3 ± 26.4	4 3	.05	+1	0.23
∞	LD _{16.8}	211.7	± 17.5	88.1 ±	6.2	123.6 ± 18.0	6 3	.08	+1	0.22
6	LDL _{16.8}	118.7	± 9.4	92.8 ±	7.4	25.9 ± 12.(0 2	.86	+1	0.23
.0	LDL _{16:8}	130.5	+ 9.8	89.1 ±	6.9	41.4 ± 12.(0 2	.97	+1	0.23
1.	LD16:8	440.3	± 56.9	115.6 ±	7.5	324.7 ± 57.'	4 2	. 29	+1	0.15
2.	LD _{16:8} (no food	1) 262.1	± 58.2	90.1 ±	8.2	172.0 ± 58.	8 1	.65	+1	0.15

Figure 1. Relation of total activity (C_0 ; mean counts/hr) to water consumption (grams/day) Y = (0.0799 ± 22.811) X + (251.361 ± 156.176) r^2 = 0.00000123 r = 0.0011073 Sy·x = 90.567





Figure 1. Relation of total activity (C_0 ; mean counts/hr) to water consumption (grams/day) Y = (0.0799 ± 22.811) X + (251.361 ± 156.176) r^2 = 0.00000123 r = 0.0011073 Sy·x = 90.567



Figure 1

Figure 2. Bar graph representing the total activity (C₀; mean counts/hr) divided by the water consumption (grams/day) during each of the 12 different periods.

Figure 3. Bar graph representing the total activity (C_O; mean counts/hr) during each of the 12 different periods.







Figure 4. Relation of total activity (2000-0400 hours) to water drinking activity (2000-0400 hours) Y = (3.589 ± 0.481) X - (420.991 ± 551.250) r²= 0.81052 r = 0.90029 Sy·x = 560.990

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Figure 4

drinking activity between 1900 and 0500 hours, during these same two periods. Linear regression analysis (Figure 5) of these data showed a highly significant correlation of ± 0.79 between the total activity (2000-0400 hours) and water drinking activity (1900-0500 hours), which is lower, however, than the first mentioned correlation. The y-intercept of the best fitting line for these data was $\pm 140.770 \pm 831.967$ which is not significantly different from zero, with a slope of 2.685 ± 0.587 , which is significantly different from zero.

These data indicate that although the daily water consumption is not related to the average hourly total activity (C_0) (Total daily activity = 24 x mean hourly total activity), the total activity during darkness is related to the water drinking activity during darkness, and the times bordering on the hours of darkness, for this particular hamster.

The Effect of Food Consumption on Water Intake

The food consumption of the hamster was measured for each of the twelve periods imposed on it. The mean food consumption per day for each of the periods was calculated and compared to the daily water consumption and the hourly drinking activity of the animal. Tables 1 and 2 shows the results.

Figure 5. Relation of total activity (2000-0400 hours) to
water drinking activity (1900-0500 hours)
Y = (2.685 ± 0.587) X - (140.770 ± 831.967)
r²= 0.61665
r = 0.78527
Sy·x = 797.932



Figure 5

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A graph (Figure 6) was prepared which plotted the food consumption (grams/day) against the water consumption data and a linear regression analysis was done to find the line which best fit the points. The line had a y-intercept of -2.37 ± 0.79 , which is significantly different from zero. This means that in the absence of food the best estimate of the water consumption (X - intercept) should be 2.81 grams per day. The measured value (period 12) was 3.55 grams per day. The slope also was significantly different from zero, having a value of 0.84 ± 0.12 . Also, there is a highly significant correlation of 0.92 between the water consumption and the food consumption of this hamster. This was expected since Fitzsimmons showed at least 70% of the total intake of water is drunk just before, during and immediately after meals, and there is furthermore a highly significant positive correlation between the amount of water associated with a particular meal and the size of that meal. These data had an r^2 value of 0.84. A bar graph of these data further illustrates this (Figure 7 and 8).

A graph (Figure 9) was also prepared which plotted the food consumption (grams/day) against the water drinking activity (C_0 ; mean counts/hour) and a linear regression analysis done. The line which best fit these points had a yintercept of 2.92 ± 1.22, which is significantly different from zero, and having a value of 0.00371 ± 0.01169 for its slope. This relationship is not significant (r = +0.0998).

Figure 6. Relation of food consumption (grams/day) to
 water consumption (grams/day)
 Y = (0.839 ± 0.115) X - (2.372 ± 0.785)
 r²= 0.84268
 r = 0.91798
 Sy · x = 0.45528





Figure 7. Bar graph representing the food consumption (grams/day) divided by the water consumption (grams/day) during each of the 12 different periods.

Figure 8. Bar graph representing the food consumption (grams/day) during each of the 12 different periods.



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Figure 9. Relation of food consumption (grams/day) to
 water drinking activity (C₀; mean counts/hour)
 Y = (0.003709 ± 0.01169) X + (2.921 ± 1.220)
 r²= 0.00997
 r = 0.09989
 Sy·x = 1.142



Figure 9

<u>Chi-square Analysis of the Interpeak Intervals</u> of Water Drinking Behavior

A preliminary chi-square analysis of the interpeak intervals was done on the activity data obtained from the animal (Appendix C). The detailed results of this analysis are found in Appendix G. It was found that there is no demonstrably significant differences in water drinking patterns (as evaluated for the observed intervals between peaks in drinking) between periods in which all the lighting regimes are the same. Consequently for period 2, 3, 4, 7, 8 and 11 ($LD_{16:8}$; lights on 0400-2000 hours) these drinking interval data were combined and used as the normal control for comparison with each of the periods with a different lighting schedule. This preliminary analysis showed that there is no demonstrably significant difference between the combined interpeak intervals from all the control periods and those with altered lighting regimes.

There is, however, a significant difference between the early control periods and the later control periods, suggesting an effect associated with aging in the animal. A chi-square analysis was done between periods 2 (12/8/75 -12/17/75) and 11 (4/9/76 - 4/18/76), both of which were control periods (Appendix G). This analysis revealed a significant difference between these two periods, even though all the conditions, except for the age of the hamster, were the same. The number of short intervals is increased and the number of long intervals is decreased in the aged hamster.
Since aging is a factor in the water drinking activity of the hamster, and it has been demonstrated that the later control periods differ significantly from the earlier control periods, it is not permissible to combine the interpeak intervals of the earlier control periods with those of the later control periods as was done in the preliminary test. Therefore, the chi-square analysis was rerun, grouping only consecutive control photoperiods $(LD_{16:8})$. This assumed that a more valid comparison can be made between neighboring periods in time, than between widely separated periods.

An analysis between period 1 $(LD_{12:12}; lights on 0600-1800)$ and periods 2, 3, and 4 (controls) did not reveal a significant different in the absolute quantity of water consumed during each of these four periods. However, the chi-square analysis between combined periods 5 and 6 $(LD_{8:4:8:4}; lights on 0200-1000 and 1400-2200 hours)$ and combined periods 2, 3, and 4 (controls) revealed a significant difference in the interpeak intervals. Yet there was no significant difference in the quantity of water consumed during each of these five periods (Table 1). However, in the grams of water consumed per kg^{3/4} of body weight periods 5 and 6 were significantly different from periods 2, 3, and 4 (Table 2).

The chi-square analysis between combined periods 9 and ¹⁰ (LDL_{16:8}; lights on 0400-2000 hours and dim light on 2000-0400 hours) and combined periods 7 and 8 (controls)

showed there was a significant difference in the interpeak intervals of the animal, between periods 9 and 10 and the control periods. Yet there was no significant difference in the quantity of water consumed during each of these four periods (Table 1), or in the grams of water consumed per $kg^{3/4}$ of body weight. (Table 2).

The chi-square analysis, between period 12 (control lighting but no food provided) and period 11 (control) showed there was no significant difference in the interpeak intervals of the animal, between period 12 and the control period. There was, however, a significant difference in the quantity of water consumed during period 11 and period 12 (Table 1). The water consumed during period 12 was about one-half of that consumed during period 11.

Interpeak intervals, for periods 5 and 6 and periods 9 and 10, was significantly different from that of their corresponding control periods. In periods 5 and 6 the time between peaks in water drinking activity tended to be shorter than time between peaks in water drinking activity of the control periods. For periods 9 and 10 just the opposite was observed. The time between peaks in the water drinking activity in periods 9 and 10 tended to be longer than the time between peaks in the water drinking activity of the control periods. However, in both of these cases the amount of water consumed was not significantly different from water consumption during the control periods. Period 12 showed no significant difference in interpeak

intervals from the control period, but the hamster consumed an amount of water which was significantly less than the amount consumed during the control period. These data point to the possible existence of at least two different neural mechanisms controlling water drinking in the hamster, one which controls drinking frequency (as indicated by interpeak interval analysis) and another which controls the amount of water consumed in a day. From the data cited, these two appear to be distinct, if not completely independent.

A chi-square analysis of the interpeak intervals was also done between the water drinking activity and the total activity peaks (Appendix D) from the same period. The results of this analysis are found in Appendix G. These results show that within a period there is no demonstrably significant difference between the interpeak intervals for water drinking activity and total activity for any of the 12 photoperiods.

II. METABOLIC EXTRAPOLATIONS OF PHYSIOLOGICAL SIGNIFICANCE (Estimated from Simple Calculations based on Empiric Equations)

Comparison of Food Consumption and Weight Gain

The hamster was young when the experiment was first started and he steadily gained weight during the first four periods. After this the animal's weight remained fairly constant until the period (period 12), during which the

animal was not supplied with food. For each of the periods the basal metabolic rate (Table 4) of the hamster was estimated using the equation:

The number of kilocalories the hamster consumed per day was also calculated (Appendix C), using 390 kcal as the metabolizable energy in 100 grams of oatmeal. The basal metabolic rate was subtracted from the kcals consumed to further estimate the residual energy available for weight gain or for activity. Table 4 shows this comparison.

In making these calculations the average weight of the hamster over the entire 10 day period was used as his weight for that period, and the average amount of food consumed each day was regarded as food intake. As can be seen from the figures, there is no demonstrable correlation between the weight gain and the food energy available for weight gain.

*often loosely referred to as the basal metabolic rate

- The effects of different photoperiods on derived indicators (computed as described in the text) Table 4.
- 1. Energy from the food consumed (kcal/day)
- 2. Mean daily standard metabolism (kcal/day)
- Kcal consumed per day minus the kcal needed for basal metabolism (kcal/day)
- 4. The mean weight gain per day (grams/day)
- 5. The food energy remaining for activity after
- the standard metabolism and weight changes are taken into account (kcal/day)

£		well area food	mean daily standard	Kcal cons- Kcal standard	mean wt.	food
гет	# DOI.	NCAL CUID/ NAY	IIIST TOOR JUI	MCT TOOP JAN	gain/day	energy left
1.	LD _{12:12}	13.31	8.05	5.26	+0.23	2.45
2.	LD _{16:8}	13.52	9.31	4.21	+0.72	-1.80
3.	LD _{16:8}	13.52	9.31	4.21	+0.72	-1.80
4.	LD _{16:8}	17.61	10.57	7.04	+0.32	4.42
5.	LDLD _{8:4:8:4}	14.61	10.57	4.04	-0.12	4.52
6.	LDLD _{8:4:8:4}	14.61	10.57	4.04	-0.12	4.52
7.	LD _{16:8}	13.18	10.43	2.75	-0.06	2.25
8.	LD _{16:8}	13.18	10.43	2.75	+0.06	2.25
9.	LDL _{16:8}	12.74	10.36	2.38	+0.17	3.11
10.	LDL _{16:8}	12.74	10.36	2.38	-0.17	3.11
11.	LD _{16:8}	15.11	10.29	4.82	+0.32	2.19
12.	LD _{16:8} (no fo	od) 0.0	9.24	-9.24	-2.11	-0.47

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The Effect of Total Activity on Food Consumption

The food consumed by an animal is used in several different ways, maintenance of the body mass, accumulation of adipose tissue and as an energy source for activity. A graph (Figure 10) was made comparing the total activity (C_0 ; mean counts/hour) to food consumption (grams/day). Linear regression analysis was done on the data points and it was found there was no correlation between the total food consumed by the animal and its total activity (r = +0.083).

The calculations were then taken a step further. The kcals needed for the BMR and those which went into adipose tissue were subtracted from the kcals contained in the food the animal consumed. In these calculations the amount of energy in adipose tissue was assumed to be 4.16 kcal/gram, with a fattening efficiency of oatmeal = 52%. The loss of adipose tissue was assumed to be 100% efficient. Table 4 gives the result of these calculations comparing the total activity (C_0 ; mean counts/hour) to the food energy (kcal) left after the BMR and fattening were subtracted from it.

From tables 4 and 3 it can be seen that there is no correlation between the total activity (or the total activity minus the water drinking activity) and the food energy available for total activity.



III. SPECTRAL ANALYSIS OF HAMSTER MOTOR AND WATER DRINKING ACTIVITY

Total Activity

In biorhythm data, statistically there tends to be a high variance between animals since individuals are frequently out of phase. This confounds the interpretation of means calculated from groups of animals, and for this reason data from only a single individual must be analyzed first. Parameters defining the activity rhythms (i.e., amplitudes, frequencies, phase angles and means) were estimated by a kind of spectral analysis often referred to as periodic regression analysis (Norton, 1975).

The value found for the major period length (τ) for the experimental periods 1, 2, 3, 4, 7, 8, 9, 10 and 11 were all slightly greater than 24 hours having a mean of 24.15 \pm 0.15 hours, and varying from 24.01 hours in period 9 to 24.44 hours in period 11. The light-dim light photoperiod in this animal did not result in the slight increase in period length observed in male hamsters kept in constant bright light (Norton, ibid). Further, harmonics of the circadian periodicity were less evident in this individual than in the animals studied by Norton (ibid).

The average phase angle (Appendix I) during the 24 hour light-dark and light-dim light photoperiods (periods 1, 2, 3, 4, 7, 8, 9, 10 and 11) was 340.6 ± 8.4 degrees with an average crest time (Appendix I) of 2242 (10:42 PM), in other words 1.31 hours before midnight, with a standard error of \pm 0.56 hours.

Significant changes in both mean activity levels (C_0) and circadian amplitude normalized to their respective means (C_{24}/C_0) were observed during periods 9 and 10 (LDL_{16:8}). Exposure to light-dim light (periods 9 and 10) resulted in a significant decrease in total activity counts (Table 3) from a mean of 287 ± 13 counts per hour for periods 1, 2, 3, 4, 7, 8, and 11 to a mean of 125 ± 9 counts per hour, and a significant decrease in normalized circadian amplitudes (C_{24}/C_0) from a mean of 1.11 ± 0.05 (periods 1, 2, 3, 4, 7, 8 and 11) to a mean of 0.538 ± 0.023 for periods 9 and 10. This effect seems to be transitory since both parameters recovered in period 11 when a control lighting regime $(LD_{16:8})$ was once again imposed.

The data from periods 5 and 6 (LDLD_{8:4:8:4}) showed no significant circadian (24 hour) peak in total activity counts (Table 3), rather a 12 hour peak was observed. The average value found for period lengths (τ) was 12.04 hours. This effect seems to be transitory since this parameter recovered in period 7 when control lighting (LD_{16:8}) was once again imposed. The average phase angle (Appendix I) occurring 1.09 hours before noon and midnight. The values of mean activity levels (C₀) for tness two periods averaged 252 ± 17 which is not significantly different from tne value of 287 ± 13 observed as the mean for periods 1, 2, 3, 4, 7, 8 and 11. The normalized 12 hour amplitude (C₁₂/C₀) were averaged and found to be 0.94 which is not significantly different from 1.11 \pm 0.05 observed as the mean for normalized circadian amplitudes from periods 1, 2, 3, 4, 7, 8 and 11.

In summary, it was observed that there were significant changes in both mean activity levels (C_0) and normalized circadian amplitudes for periods 9 and 10 from the control values. Periods 5 and 6 showed a 12 hour peak rather than a 24 hour peak. Other than these observations, the other parameters from period 5, 6, 9 and 10 were not significantly different from those of the control periods.

Water Drinking Activity

The observed mean value for \mathcal{T} in the analysis of water drinking during periods 1, 2, 3, 4, 7, 8, 9, 10 and 11 is 23.97 \pm 0.22 hours, varying from 23.47 hours for period 10 to 24.31 hours for period 1. During the light-dim light photoperiods (periods 9 and 10) a slight decrease in the period length for water drinking activity was observed. This decrease was most pronounced in the second 10-day light-dim light period (period 10). This effect seems to be trnasitory since the period length recovered in period 11 when a control lighting regime (LD_{16:8}) was once again imposed.

The average phase angle (Appendix I) during periods 1, 2, 3, 4, 7, 8, 9, 10 and 11 was 320.7 ± 30.5 degrees

with an average crest time (Appendix I) of 2123 (9:23 PM), in other words 2.62 hours before midnight.

No significant change in mean water drinking activity levels (C₀) were observed during periods 9 and 10 (LDL_{16:8}), however, period 1 had a count twice as high as the average. The average of the mean water drinking activity levels for periods 2, 3, 4, 7, 8, 9, 10 and 11 was 93 ± 4, and that for period 1 was 190 ± 17. A significant change in the normalized circadian amplitudes (C₂₄/C₀) was observed during period 9 and 10 (LDL_{16:8}).

Exposure to light-dim light (periods 9 and 10) resulted in a significant decrease in normalized circadian amplitudes (C_{24}/C_0) from a mean of 0.685 ± 0.030 (periods 1, 2, 3, 4, 7, 8 and 11) to a mean of 0.367 ± 0.016 for periods 9 and 10. This decrease was again most pronounced in the second 10-day light photoperiod (period 10). This effect was reversed only partially in period 11 when a control lighting regime (LD_{16:8}) was again imposed. The C_{24}/C_0 value for period 11 was 0.405 which is well below the average.

The data from periods 5 and 6 (LDLD_{8:4:8:4}) showed no significant 24 hour peak in water drinking activity counts (Table 3). Rather, a 12 hour peak was observed. The average value found for period length (τ) was 12.03. The disappearance of the circadian period seems to have been transitory since it reappeared in period 7 when control lighting (LD_{16:8}) was imposed. The average phase angle (Appendix I) was 351.8 degrees having an average crest time (Appendix I) occurring 1.08 hours before noon and midnight. The values of mean water drinking activity levels (C_0) for these two periods averaged 92 ± 5 which is not significantly different from the value of 93.4 observed as the mean for periods 2, 3, 4, 7, 8, 10 and 11. The normalized 12 hour amplitude (C_{12}/C_0) were averaged and found to be 0.653 which is not significantly different from 0.695 observed as the mean for normalized circadian amplitudes from periods 1, 2, 3, 4, 7, 8 and 11.

Water drinking activity in periods 9 and 10 (LDL_{16:8}) showed significant peaks in period lengths (7) other than the 24 hour peak. In period 9 a significant 2.60 hour peak in water drinking activity was observed. In period 10 several significant peaks in water drinking activity were observed, these were 30.43 hours, 25.65 hours, 13.09 hours, 4.50 hours and 2.86 hours.

In summary it should be noted that period 1 had a C_0 value significantly greater than the control periods. Periods 5 and 6 had a 12 hour peak, not the circadian rhythm observed in the control periods. Periods 9 and 10 showed a slight decrease in period length, and a significant decrease in the c_{24}/C_0 ratio. Also, in periods 9 and 10 significant period lengths other than the 24 hour circadian peak were observed. All the parameters, except the C_{24}/C_0 ratio (Periods 9 and 10), returned to control levels when a control lighting regime (LD_{16:8}) was once again imposed.

DISCUSSION

In this study a young male hamster's total activity, water drinking activity, water consumption food consumption and weight changes were recorded for twelve 10-day periods. The conditions during these periods were altered in different ways (mainly by changing the light-dark photoperiods) and the effects these altered conditions had on the total activity, water drinking activity, water consumption, food consumption and weight of the hamsters were noted. To help clarify this discussion two tables were prepared (Tables 5 and 6) from the data.

The first calculations of food and water consumption (Table 1) revealed few things of interest and generally no trends. However, when water consumption, total available water and food consumption were corrected to body weight to the 3/4 power (giving an approximation of surface area) certain trends do show up. The most outstanding observation is the effect time had on these parameters. The effect which aging had on the hamster was determined by running a linear regression analysis of these parameters against time. A listing of the results follows.

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Table	

- 1. Water consumption $(g/kg^{3/4}/day)$
- 2. Milligrams of water per lick
- Water drinking activity mean interpeak intervals (hours) 3.
- 4. C_{24}/C_0 ratio for water drinking activity
- Phase angle for water drinking activity (degrees) 5.
- 6. Period length for water drinking activity (hours)
- for each of the 12 photoperiods.

жч	perimental eriod #	Water Cons. (g/kg ³ /4/day)	Water/lick (milligrams)	Water Drink. Act. Interpeak Intervals	Water D C ₂₄ /C ₀	rinking / degrees)	Activity イ (hours)
1	LD _{12:12}	60.7	1.54 ± 0.13	3.24	0.803	322.5	24.31
7	LD _{16:8}	54.3	3.58 ± 0.30	3.20	0.686	335.2	24.06
З	LD _{16:8}	54.3	3.52 ± 0.33	3.08	0.837	328.8	24.05
4	LD _{16:8}	54.6	3.52 ± 0.28	3.08	0.830	354.4	24.05
S	LDLD _{8:4:8:4}	52.1	3.34 ± 0.26	2.76	*0.658	16.3	11.97
9	LDLD _{8:4:8:4}	52.1	3.80 ± 0.30	2.96	*0.792	327.4	12.09
7	LD16:8	43.7	3.05 ± 0.23	3.24	0.669	317.4	24.01
ø	LD16:8	43.7	3.08 ± 0.22	3.00	0.637	333.3	23.92
6	LDL _{16:8}	43.2	2.86 ± 0.23	3.29	0.442	331.6	23.90
10	LDL _{16:8}	43.2	2.97 ± 0.23	2.70	0.291	245.1	23.47
11	LD _{16:8}	43.4	2.29 ± 0.15	2.96	0.405	318.2	23.98
12	LD _{16:8} (no f	:00d 27.1	1.64 ± 0.1 5	4.44			
	*c ₁₂ /c ₀						

- A comparison of different indicators relating to total activity Table 6.
- 1. Total water available $(g/kg^{3/4}/day)$
- 2. Total activity mean interpeak intervals (hours)
- 3. C_{24}/C_0 ratio for total activity
- 4. Phase angle for total activity (degrees)
- 5. Period length for total activity (hours)
- for each of the 12 photoperiods.

ExI	perimental Period #	Tot. Ayail. Water (g/kg ³ /4/day)	Total Activity Interpeak Intervals	Total A C ₂₄ /C ₀	ctivity degrees)	r (hours)
	LD _{12:12}	78.5	3.04	0.992	322.9	24.17
7	LD _{16:8}	70.0	3.24	1.050	346.7	24.13
ы	LD _{16:8}	70.0	2.86	1.255	331.3	24.09
4	LD _{16:8}	72.8	3.24	1.187	347.9	24.09
S	LDLD8:4:8:4	67.1	2.96	*0.967	355.8	12.03
9	LDLD _{8:4:8:4}	67.1	3.00	*0.917	350.2	12.05
7	LD _{16:8}	57.3	3.12	1.089	347.4	24.05
œ	LD _{16:8}	57.3	3.00	1.087	345.1	24.03
6	LDL _{16:8}	56.6	3.16	0.501	340.2	24.01
10	LDL _{16:8}	56.6	2.79	0.574	341.8	24.37
11	LD _{16:8}	59.3	2.58	1.085	341.7	24.44
12	LD _{16:8} (no fo	od) 44.3	3.75			

*c₁₂/c₀

r = 0.671 (significant)

From the observations, it is suggested that the regulation of drinking has reference points adjusted to the relative surface area, to food intake and probably to changes in body composition. It is also suggested that these physiological variables can be altered both by age of the animal and by exposure to different lighting schedules.

A fourth parameter which has shown to be related to time, by linear regression analysis, was the circadian period length (\mathcal{T}) of the water drinking activity data. As time passed, the period length (\mathcal{T}) decreased. The results of the linear regression analysis follows. 1. Relation of water drinking activity period length (γ , in hours), Y, to time (periods 1-11),X. Y = (-0.042 ± 0.016)X + (24.24 ± 0.11) r^2 = 0.498 r = 0.706 (significant)

A chi-square analysis was done between periods 2 (12/8/75 - 12/17/75) and 11 (4/19/76 - 4/18/76), both of which were control periods. This analysis revealed a significant difference between these two periods, even though all the conditions, except for the age of the hamster, were the same. The number of short intervals is increased and the number of long intervals is decreased in the aged hamster.

Isolation for a hamster is an abnormal state and at this point it may be worth noting that the isolation the hamster experienced during the course of the experiment could have an effect on many of the parameters measured. Chi-square analysis of the interpeak intervals between periods 2 and 11 showed these two periods differed a great deal even though both periods had control lighting regimes These two periods were separate in time by about $(LD_{16\cdot 8})$. three months, which is a fairly short period of time. It is questionable whether aging along can cause this large change. It may be more reasonable to assume that a combination of aging, isolation and past lighting history causes this change. Linear regression analysis has shown that changes in water consumption $(g/kg^{3/4}/day)$, total available

water $(g/kg^{3/4}/day)$, food consumption $(g/kg^{3/4}/day)$ and the water drinking activity period length (7) can be observed as a function of time. These changes, though not as great as the change observed in the interpeak interval data, can probably also be attributed to the combined effect of aging, isolation and past lighting history. The effect of isolation could be corrected by placing two hamster in one cage. The combination of the parameters for two hamsters, however, would tend to confound the interpretation of the data.

The average interpeak intervals for water and drinking activity and total activity are listed in Tables 5 and 6. The average interpeak intervals for water drinking activity (periods 1-11) is 3.00 ± 0.06 , with an average interpeak interval for total activity (periods 1-11) of 3.05 ± 0.06 . The existence of these 3 hour peaks in the interpeak interval data points to the existence of a rhythm, other than the circadian rhythm. In fact, the total disappearance of the circadian rhythm in periods 5 and 6, puts its actual existence in question. Even when the circadian rhythm disappears, however, these 3 hour interpeak intervals remain. These 3 hour peaks in water drinking activity are especially interesting since urination in rats produces a periodicity of about 3-4 hours (Richter, 1965). Since water drinking activity and urination are the two major parameters involved in water regulation in the body, the same or similar neural mechanisms may be involved in controlling both of them.

These 3 hour interpeak intervals for water drinking activity and total activity are probably superimposed on the circadian rhythm. During darkness the 3 hour interpeak intervals probably occur at a raised level. During the hours of light the 3 hour interpeak intervals probably occur at a lower level. This would produce a figure as follows.



The total activity seems to be controlled better by circadian rhythms than the water drinking activity is. This idea is supported by the fact that the C_{24}/C_0 ratio, for total activity is significantly larger than that for water drinking activity (Tables 5 and 6). Also, in periods 5 and 6, 29.7% of the water drinking activity counts occurred during the 4 hours of darkness surrounding midnight, 40.9% of the total activity counts occurred during these same 4 hours. Only 22.2% of the total activity counts occurred during the 4 hours of darkness surrounding noon and 20.4% of the water drinking activity counts occurred during these same 4 hours. Even though the peak at midnight was greater, time series analysis showed no sifnificant 24 hour peak. Also, for water drinking activity the phase angle (\neq) and crest time (C.T.) were much more variable than those observed in the total activity data (Tables 5 and 6). In periods 9 and 10 (LDL_{16:8}) the water drinking activity data showed several peaks other than the 24 hour peak. This is probably caused by a dissociation of circadian rhythm, seen in contant light (Norton, 1975).

The quantity of water consumed during periods 5, 6, 9 and 10 remained essentially the same as that consumed during the control periods. However, when a chi-square analysis was run on the interpeak intervals, it was found that these four periods did not at all resemble the control periods. This seems to indicate the possible existence of two different neural mechanisms controlling the water intake of the hamster, one which controls how much he drinks and one controlling how often he drinks. This idea was further supported when a chi-square analysis of the interpeak intervals from period 12 was run. The animal was kept on a control lighting regime $(LD_{16:8})$, but no food was supplied to the animal so its water consumption would decrease. Period 12 was just the opposite of periods 5, 6, 9 and 10. The interpeak interval data from this period resembled that of the control periods, however, the quantity of water consumed during period 12 was significantly different from the quantity of water consumed during the control periods.

A study by Stetson (1976), on the hamster, shows that the suprachiasmatic nuclei may be a primary oscillatory regulating a variety of rhythms, or a coupled oscillator in

in a two-or multioscillator system. This may possibly be the neural mechanism controlling how often the animal drinks. The quantity of water consumed is probably controlled by the hypothalamus through a combination of cellular and extracellular mechanisms.

Fitzsimmons and LeMagnen (1969) showed that in the rat there was a very close temporal relationship between eating and drinking. At least 70% of the total intake of water is drunk just before, during and immediately after meals. The close relationship of eating and drinking was observed in this study also. There was a highly significant positive correlation between the average daily food consumption and the average daily water consumption. This foodassociated drinking may be a learned response based on previous experience of the dipsogenic properties of the diet which enable an animal under stable conditions to anticipate its future water requirements by drinking appropriate amounts of water at meal time. Starvation (period 12) of the hamster caused a significant change in water consumption, but it had little affect on the total activity or on the water drinking activity.

Changing the light-dark photoperiods had the greatest effect on the total activity of the animal. During the control periods $(LD_{16:8})$ the animal showed only one peak in total activity, that occurring at or around midnight. While being subjected to the $LDL_{16:8}$ photoperiod (Periods 9 and 10) the hamster had only one peak in total activity that

occurred around midnight, however, the mean total activity counts per hour decreased to about one-half as great as the control periods. This was expected since Norton (1975) showed that hamster subjected to constant light had a significant decrease in total activity. The C_{24}/C_0 ratio also decreased significantly in periods 9 and 10. This suggests that several periodicities may be present whose period lengths are near 24 hours. Dissociation of these periodicities could cause a reduction in the C_{24}/C_0 ratio of the single 24 hour peak observed in periods 9 and 10 (LDL_{16.8}).

It was observed that there is some variation in the different parameters studied. From the observations, it is suggested that the regulation of drinking has reference points adjusted to food intake and probably to changes in body composition. Food consumption is probably regulated by changes in body composition also. Water consumption, total available water and food consumption $(g/kg^{3/4}/day)$ tend to decrease as the hamster ages. However, the physiological needs of the animal must be met. Therefore these data suggest that the physiological requirements of the animal (food and water) can be altered by the age of the animal and by exposure to different lighting schedules. Thev also suggest that aging of the animal causes a decreased need for food and water.

SUMMARY AND CONCLUSIONS

Lighting regimes different from normal control
lighting (LD_{16:8}) seems to cause a variation from normal in
the water consumption of the animal.

2. Chi-square analysis of the interpeak interval data, compared to the water consumption, seems to show the possible existence of two different neural mechanisms, one contorlling the frequency of water drinking and the other controlling the amount of water consumed.

3. The daily water consumption is not related to the average hourly total activity (C_0) , however, the total activity is related to the water drinking activity during the hours of darkness, and to those bordering darkness, for this particular hamster.

4. There was a highly significant positive correlation between the water consumption and the food consumption of the hamster during each of the periods. This is expected since approximately 70% of all water drinking is associated with eating (Fitzsimmons and LeMagnen, 1969).

5. There is no demonstrable correlation between the weight gain of the hamster and the food energy available for weight gain.

6. There is no correlation between the food consumed by the animal and its total activity.

7. Water drinking activity and total activity both show an average interpeak interval time of approximately 3 hours. These 3 hour peaks are probably superimposed on the circadian rhythm.

8. Spectral analysis of periods 5 and 6 show a total disappearance of the circadian rhythm. A significant 12 hour peak replaces it.

9. The decreased C_{24}/C_0 ratio observed in periods 9 and 10 (LDL_{16:8}) may be due to the dissociation of several periodicities which have 24 hour peaks.

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APPENDICES

APPENDIX A

Total water consumption Tot. $H_2^0 = H_2^0$ intake + Food H_2^0 + Metabolic H_2^0 + H_2^0 from fat loss H_2^0 formed from different foodstuffs (Schmidt - Nielsen, 1964) Food type Grams of metabolic H_2^0 formed

		2 rormer
		per gram of food metabolized
	Starch	0.556 grams
	Fat	1.071 grams
	Protein	0.396 grams
1	composition	(100 grams) (Best - Tavlor, 1966)

Oatmeal composition (100 grams) (Best - Taylor, 1966)

H₂O (%) Carbohydrate (gm) Fat (gm) Protein (gm) 8.3 69.4 7.4 14.2

Water from 100 grams of oatmeal

Free H ₂ 0	8.3 grams
From Protein	5.623 grams
From Fat	7.925 grams
From Carbohydrate	38.586 grams
Total H ₂ 0	60.434 grams
_	H ₂ O/100 gms. food

APPENDIX B

Sample calculation of the standard metabolism for the hamster.

Weight of hamster W = 80 grams $W^{3/4}(Kg) = 0.150$ $B = 70 W^{3/4}$ B = 70 (0.150)B = 10.6 kcal

Standard metabolism for an 80 gram hamster

B = 10.6 kcal

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(all values are one hour apart; read horizontally, 2 lines equal 24 hours) Sample data to determine how interpeak intervals may be determined.

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							rity	of activ	peaks (*	
0.	.0	314.*	0.	116.*	0.	.0	0.	272.*	186.	56.	0.
129.*	0.	226.*	0.	183.	252.*	0.	0.	0.	.0	0.	0.
0.	212.*	0.	371.*	0.	0.	.0	2.	249.*	0.	133.*	0.
319.*	259.	0.	186.	497.*	181.	295.*	2.	0.	0.	104.*	.0
0.	181.*	106.	0.	247.*	0.	0.	0.	458.*	0.	.0	.0
0.	183.*	181.	169.	225.	359.*	108.	2.	0.	0.	102.	129.*
0.	0.	0.	108.	274.*	0.	.0	512.*	0.	0.	225.*	.0

can be read from these data:

6 hrs, 4 hrs, 5 hrs, 4 hrs, 3 hrs, 4 hrs, 2 hrs, 2 hrs, 5 hrs, 2 hrs, 4 hrs, 4 hrs, 2 hrs, 2 hrs. 5 hrs, 2 hrs, 2 hrs, 3 hrs, 4 hrs, 3 hrs, 3 hrs, 3 hrs, 8 hrs,

APPENDIX C; concluded

This gives the following observed frequencies for individual intervals:

1 case 8 hrs 0 cases 1 case 7 hrs 6 hrs 6 cases 3 cases 5 hrs 4 hrs 6 cases 5 cases 2 hrs 3 hrs

Similarly, there are no fractional frequencies of cases. Since the word "frequency" Note that intervals less than 2 hours are indeterminate and is used in two senses in this thesis, care must be exercised in that all values are whole numbers (digits) or zero. following the descriptive implications.

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Frequencies of water drinking activity interpeak intervals, per 10-day

experimental period.

	١٢	3 hr	4 hr	5 hr and more	Total peaks	Mean calc. periodicity (
	4	27	13	10	74	3.24
	2	35	œ	10	75	3.20
	8	31	11	ø	78	3.08
	32	26	11	Q	78	3.08
	11	33	6	4	87	2.76
-	41	23	6	δ	81	2.96
	29	19	16	10	74	3.24
	44	18	6	6	80	3.00
	23	29	10	11	73	3.29
	37	32	13	7	89	2.70
	39	21	13	8	81	2.96
	23	11	14	6	54	4.44
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Frequencies of total activity interpeak intervals, per 10-day

experimental period.

Period #	2 hr	3 hr	4 hr	5 hr and more	Total peaks	Mean calc. periodicity (T
1	34	24	14	7	79	3.04
2	21	31	13	6	74	3.24
ъ	37	32	6	9	84	2.86
4	30	29	11	4	74	3.24
S	35	28	11	7	81	2.96
Q	35	25	13	7	80	3.00
7	30	27	14	6	77	3.12
œ	42	18	6	11	80	3.00
6	28	28	6	11	76	3.16
10	38	28	6	11	86	2.79
11	43	30	16	4	93	2.58
12	24	16	17	7	64	3.75

APPENDIX E

Sample Chi-square analysis

interpe	eak	Pd 9	Pd 10	Tot	als
interva	1			obs.	x ²
	observed	23	37	60	
	calculated	27.0	33.0		
2 hr	(obscalc.)	-4.0	4.0		
	$(obscalc.)^2$	16.00	16.00		
(obs	calc.) ² /calc.	0.593	0.485		1.078
		29	32	61	
		27.5	33.5		
3 hr		1.5	-1.5		
		2.25	2.25		
		0.082	0.067		0.149
		10	13	23	
		10.4	12.6		
4 hr		-0.4	0.4		
		0.160	0.16		
		0.015	0.013		0.028
5 hr and	above	11	7	18	
		8.1	9.9		
		2.9	-2.9		

APPENDIX E; concluded

	8.41	8.41		
	1.038	0.849		1.887
Totals	73	89	162	3.142
degrees of freedo	om = 3			
P = 0.50 (3.142)	0.30 (see	footnote	e)	
There is no signi	ficant di	fference	between	periods
and 10.				

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footnote: this line is to be read as follows:

"The probability of finding a X^2 value of 3.142 lies between 0.50 and 0.30." This practice is followed throughout the succeeding X^2 tables.

APPENDIX F

HP-65 LINEAR REGRESSION PROGRAM (Stat 1-22A)

This program must be used in conjunction with Stat 1-05A, Sums for Two Variables, to fit a straight line

$$y = a_0 + a_1 X$$

to a set of data points $\{(x_i, y_i), i = 1, 2, ..., n\}$ by the least squares method.

The program computes:

1. regression coefficients a_0, a_1

$$a_{1} = \frac{\sum x_{i}y_{i} - \frac{\sum x_{i}\sum y_{i}}{n}}{\sum x_{i}^{2} - \frac{(\sum x_{i})^{2}}{n}}$$
$$a_{0} = \overline{y} - a_{1}\overline{x}$$

where

$$\overline{x} = \frac{\Sigma x_i}{n}$$
$$\overline{y} = \frac{\Sigma y_i}{n}$$

APPENDIX F; continued

2. coefficient of determination

$$r^{2} = \frac{\left[\sum_{i} x_{i} y_{i} - \frac{\sum_{i} \sum_{j} y_{i}}{n}\right]^{2}}{\left[\sum_{i} x_{i}^{2} - \frac{\left(\sum_{i}\right)^{2}}{n}\right] \left[\sum_{i} y_{i}^{2} - \frac{\left(\sum_{i}\right)^{2}}{n}\right]}$$

 r^2 can be interpreted as the proportion of total variation about the mean \overline{y} explained by the regression. In other words, r^2 measures the "goodness of fit" of the regression line. Note that $0 \le r^2 \le 1$, and if $r^2 = 1$, we have a perfect fit.

3. estimated value \hat{y} on the regression line for any given \boldsymbol{x}

 $\hat{y} = a_0 + a_1 x$

4. standard error of estimate of y on x

$$Sy \cdot x = \sqrt{\frac{\Sigma (y_i - \hat{y}_i)^2}{n - 2}}$$

$$= \sqrt{\frac{\Sigma y_{i}^{2} - a_{0}\Sigma y_{i} - a_{1}\Sigma x_{i}y_{i}}{n - 2}}$$

5. standard error of the regression coefficient a_0

$$S_{0} = S_{y \cdot x} / \frac{\sum_{i=1}^{\sum_{i=1}^{n} 2}}{n \left[\sum_{i=1}^{\sum_{i=1}^{n} 2} - \frac{(\sum_{i=1}^{n} 2)^{2}}{n} \right]}$$

6. standard error of the regression coefficient a_1



Note: n is a positive integer and $n \neq 1$ or 2.

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APPENDIX G

Results of chi-square analysis

Period #	Conditions	D.F.	x ²	Pnull
2vs	LD16:8			
3vs	^{LD} 16:8			
4vs	^{LD} 16:8	15	15.397	0.50 (16.397) 0.30 Not significant
7vs	^{LD} 16:8			
8vs	^{LD} 16:8			
11	LD _{16:8}			
2v s	^{LD} 16:8	3	9.412	0.025 (9.412) 0.01 Significant Period 2 has a
11	LD16:8			mean length be- tween peaks which is longer than in period 11.
2vs	^{LD} 16:8			
3v s	LD16:8	6	1.746	0.95 (1.746) 0.90 Not significant
4	^{LD} 16:8			
7vs	^{LD} 16:8	3	4.933	0.20 (4.933) 0.10 Not significant
8	LD _{16:8}			
5vs	^{LD} 8:4:8:4	3	3.607	0.50 (3.60) 0.30 Not significant
6	^{LD} 8:4:8:4			

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Period 9vs 10 2,3, 5,6 2,3 9,7 2

APPENDIX G; continued

	Conditions	D.F.	x ²	Pnull
Period #		3	3.142	0.50 (3.142) 0.30
9vs	LDL 16:8	-		Not significant
10	LDL16:8			
2,3,4,7,8,11v	s ^{LD} 16:8	3	5.471	0.20 (5.47) 0.10 Not significant
5,6	^{LD} 8:4:8:4			
2,3,4,7,8,11v	vs ^{LD} 16:8	3	2.864	0.50 (2.864) 0.30 Not significant
9,10	LDL16:8			
2,3,4,7,8,11	vs ^{LD} 16:8	3	4.640	0.30 (4.640) 0.20 Not significant
12	^{LD} 16:8 ^{(no}	food)		
lvs	^{LD} 12:12	3	0.606	0.90 (0.606) 0.80 Not significant
2.3.4	^{LD} 16:8			
2,3,4vs	LD _{16:8}	3	6.751	0.10 (6.751) 0.05 "Significant" In periods 5 and
5,6	LD _{8:4:8:4}			6 the time be- tween peaks tend- ed to be shorter than the control.
7 8vs	LD _{16:8}	3	7.109	0.10 (7.109) 0.05 "Significant
9,10	LDL _{16:8}			In periods 9 and 10 the time be- tween peaks tend- ed to be longer than the control.

Perio 11vs 12 1. 2. 3. 4

APPENDIX G; concluded

Pei	iod #	Conditions	D.F.	x ²	
11	lvs	^{LD} 16:8	3	2.268	0.7 (2.268) 0.50
12	2	LD _{16:8} (no for	od)		Not significant
1.	T.A. vs W.D.A.	^{LD} 12:12	3	2.340	0.7 (1.536) 0.5 Not significant
2.	T.A. vs W.D.A.	LD _{16:8}	3	1.536	0.7 (1.578) 0.5 Not significant
3.	T.A. vs W.D.A.	LD _{16:8}	3	1.578	0.7 (1.578) 0.5 Not significant
4.	T.A. vs W.D.A.	^{LD} 16:8	3	2.001	0.7 (2.001) 0.5 Not significant
5.	T.A. vs W.D.A.	LDLD _{8:4:8:4}	3	1.708	0.7 (1.708) 0.5 Not significant
6.	T.A. vs W.D.A.	LDLD ₈ :4:8:4	3	1.475	0.7 (1.475) 0.5 Not significant
7.	T.A. vs W.D.A.	^{LD} 16:8	3	2.501	0.5 (2.501) 0.3 Not significant
8.	T.A. vs. W.D.A.	^{LD} 16:8	3	0.246	0.98 (0.246) 0.95 Not significant
9.	T.A. vs W.D.A.	LDL16:8	3	0.511	0.95 (0.511) 0.90 Not significant
10.	T.A. vs W.D.A.	LDL _{16:8}	3	1.149	0.80 (1.149) 0.70 Not significant
11.	T.A. vs W.D.A.	^{LD} 16:8	3	2.571	0.50 (2.571) 0.30 Not significant
12.	T.A. vs W.D.A.	LD 16:8 ^{(no for}	od) 3	0.492	0.95 (0.492) 0.90 Not significant
vs	E 110mau				

vs = versus

Under Pnull, the Pnull value to the left and the right of the E X^2 are the values between which the actual Pnull value lies. These can be read as indicated earlier (footnote, Appendix E).

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APPENDIX H

Spectral analysis values for the amplitude of the 24 hour peak found in total activity and water drinking activity.

Period #	C ₂₄ (Tot.a	act.)	C _a (Wa	t.D	rink)	T./	۹.
	24		24			^c 24 ^{(minus}	W.D.A.)
Hamster #1							
Days 1-10	458	± 50	39	± 11	L	421	
1-15	395	± 43	32	± 9		363	
Hamster #2							
1	341	± 26	152	± 1!	5	189	
2	222 :	± 18	58	± 7		164	
3	327 =	± 45	72	± 7		255	
4	298 :	± 21	81	± 7		217	
*5	*262	± 24	*64	± 7		*198	
*6	*226 =	± 15	*68	± 6		*158	
7	315 =	± 21	60	± 6		255	
8	230	± 14	56	± 6		174	
9	69 =	± 9	41	± 7		19	
10	75 :	± 9	26	± 7		49	
11	478 :	± 53	4 7	± 7		431	

*Periods 5 and 6 values are taken from the 12 hour peak occurring in these periods.

shown in Al	ppendix H.	,				
Period #	Total Ø24	Activity C.T. ₂₄ (hr of dav)	Water Ø ₂₄ (degrees)	Drinking Act. C.T. ₂₄ (hr of day)	T.A. mi Ø ₂₄ (degrees)	nus W.D.A. C.T. ₂₄ (hr of day)
lamster #1	(coolgon)					
Davs 1-10	20.1	1.34	328.7	22.26	51.4	-20.92
Days 1-15	20.8	1.39	342.3	22.87	38.5	-21.48
lamster #2						
1	322.9	21.68	322.5	21.78	0.4	-0.10
- ~	346.7	23.23	335.2	22.40	11.5	0.83
טי ו	331.3	22.17	328.8	21.96	2.5	0.19
4	347.9	23.27	354.4	23.67	-6.5	-0.40
* 5	*355.8	*11.90	*16.3	*0.54	*-20.5	*11.36
* 6	*350.2	*11.72	*327.4	*10.99	*22.8	*0.73

APPENDIX I

Phase angles (¢) and crest time (C.T.) for the 24 hour peaks previously

96

APPENDIX I; concluded

2.01	23.5	21.19	318.2	23.20	341.7	11
7.16	96.7	15.98	245.1	23.14	341.8	10
0.68	8.6	22.01	331.6	22.69	340.2	6
06.0	11.8	22.14	333.3	23.04	345.1	œ
2.04	30.0	21.17	317.4	23.21	347.4	7
W.D.A.	T.A. minus	Drinking Act.	Water	Activity	Total	

*These values are taken from the 12 hour peak occurring in periods

5 and 6.









