CIRCADIAN PHASE DEPENDENT EFFECTS OF D-AMPHETAMINE SULFATE AND LEVEL OF DEPRIVATION ON WATER INTAKE IN HOODED RATS

by

Anthony John Golden

Thesis submitted to the Graduate Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
in
Psychology

APPROVED:

R. A. Hughes, Chairman

J. J. Franchina

W. B. Pavlik

J. A. Sgro

September, 1973

Blacksburg, Virginia

ACKNOWLEDGMENTS

The author wishes to express his indebtedness to Dr. R. A. Hughes for chairing his committee and for his consistently valuable help and advice in the completion of this work. The author is also very grateful to Dr. J. J. Franchina, Dr. W. B. Pavlik and Dr. J. A. Sgro, for sitting on his committee. A special thanks is in order to who aided in the procurement of items needed to conduct this research.

LIST OF FIGURES

TABLE OF CONTENTS

INTRODUCTION

The term "circadian" has been used to identify those behaviors which exhibit a 24 hour cyclic pattern. Behavioral research into the nature of these cycles has been based primarily upon three parameters: activity (A. Cone, 1970; D. Cone, 1970; Cone & Cone, 1968, 1969a, 1969b; Evans, 1971; Hobbs, Miller, Bunnell & Peacock, 1972; Lord, 1964; Quay, 1971); food intake (Booth, 1972; Evans, 1971; Fitzsimons, 1957; Siegel & Stuckey, 1947; Zucker, 1971); and water intake (Cone, Cone, Golden & Sanders, 1973; Cone & Golden, 1971, 1972, 1973; Fitzsimons, 1957; Holdstock, Blesovsky & Verschoor, 1972; Oatley, 1967a, 1967b; Siegel & Stuckey, 1947; Young & Richey, 1952; Zucker, 1971).

Early research on patterns of water intake in rats defined a basic nocturnal pattern with the largest proportion of the intake occurring during the first six hours of the dark period (Siegel & Stuckey, 1947) with peak drinking occurring approximately midway through the dark period (Young & Richey, 1952). More recently, Cone and Golden (1971, 1972) have utilized a continuous monitoring technique to conduct a systematic examination of the development of water intake cycles from the 20th to the 120th day of age, using hooded and albino rats of both sexes. Their data indicate the presence of a reliable

circadian cycle of water intake in all subjects throughout the age range examined. There were, however, significant differences in the temporal distribution of intake as a function of both the age and the strain of the organism. Hooded rats exhibited the most stable temporal pattern of intake with a single, distinct peak midway through the dark period. Albino rats exhibited considerable age dependent variability in their temporal pattern of intake with the adult males exhibiting a series of three distinct nighttime peaks. While indicating the presence of a reliable circadian rhythm in both strains studied, these findings indicate that adult male hooded rats may be the most desireable subjects for research in this area.

Although considerable research has been conducted into the neurological regulation of water intake, few, if any, systematic examinations have been made of cyclic alterations in the activity of these specific neural substrates. Early research (Andersson & McCann, 1955; Fisher & Coury, 1964; Levitt & Fisher, 1966) concentrated primarily upon the role of cholinergic systems in the regulation of water intake. More recent research, however, indicates that the major regulator of "free-running" water intake is an adrenergic system (Blass & Chapman, 1971; Krikstone & Levitt, 1970). The role of cholinergic

systems appears to be relatively minor under "free-running" conditions (Blass & Chapman, 1971) but assumes increasing importance in the regulation of intake following both water deprivation and systemic injections of hypertonic solutions (Block & Fisher, 1970).

Evidence for the existence of an adrenergic system for controlling "free-running" water intake comes from research involving the pre-optic area of the hypothalamus. Intracranial adrenergic stimulation of the pre-optic area with norepinephrine has been shown to increase water intake in non-deprived rats and to decrease water intake in water deprived rats (Hutchinson & Renfrew, 1967). This finding would indicate that the pre-optic area may have its greatest influence following very short periods of deprivation such as those reported to occur naturally in the "free-running" animal (Cone & Golden, 1971, 1972). It has further been demonstrated that lesions which involve the pre-optic area significantly reduce organismic sensitivity to experimentally induced cellular dehydration (Blass, 1968), the type of dehydration which would normally occur following short periods of water deprivation (Hsaio & Trankina, 1967). These same lesions produce no change in organismic sensitivity to extra-cellular dehydration (Blass, 1968), the type of dehydration which becomes prevalent following water deprivation periods

of over 22 hours (Hsaio & Trankina, 1967).

These findings lend considerable support to the position that an adrenergic system is involved in the maintainance of fluid balance during the cellular dehydration phase of short term water deprivation. This position also conforms to the type of regulatory system proposed by some researchers in order to account for "free-running" water intake (Blass & Chapman, 1971; Krikstone & Levitt, 1970). The interaction between adrenergic stimulation and level of deprivation reported by Hutchinson and Renfrew (1967) further suggests that water intake following relatively short periods of deprivation is determined to at least some extent by the availability of adrenergic transmitter substances in the brain.

A definite circadian cycle has been demonstrated in both the brainstem production of the adrenergic transmitter noradrenalin (Jouvet, 1967) and its corresponding concentration in brain tissue (Scheving, Harrison, Gordon & Pauly, 1968). Based upon the interaction reported by Hutchinson and Renfrew (1967), these naturally occurring circadian fluctuations in the availability of an endogenous adrenergic transmitter would be expected to produce corresponding fluctuations in the sensitivity of the organism to cellular dehydration. This effect may serve as a partial basis for the observed circadian rhythm of

"free-running" water intake. Further evidence in support of this position may be seen in the large degree of similarity between the series of nighttime peaks in brain norepinephrine content reported by Scheving, et. al. (1968) for adult male albino rats and the nighttime pattern of water intake reported by Cone and Golden (1971) for similar subjects. Research conducted by Bolles (1965) and Oatley (1967a) which indicates that post-deprivation water intake is not independent of the organism's circadian phase at the time of deprivation lends some additional support to this view.

In addition to natural organismic variations, several methods may be used to artificially manipulate the amount of available adrenergic transmitter substances in the brain. One of these methods is to administer systemic injections of d-Amphetamine sulfate which has been shown to stimulate the release of endogenous norepinephrine in brain (Carr & Moore, 1969; Stein & Wise, 1969). Although a surgical procedure would provide the greatest amount of information concerning specific neural substrates involved in the drinking process, the use of systemic injections of d-Amphetamine sulfate is preferred to surgical intervention for several reasons. Since, in the present framework, surgical intervention would involve the pre-optic area, the effects of such a manipulation would

include severe impairment of body temperature regulation, adipsia, shivering and the eventual death of the subject (Andersson, Gale, Hokfelt & Larsson, 1965). Although these effects may be moderated by controlling the extent of the damage (Andersson, Gale, Hokfelt & Larsson, 1965) and localized heating and cooling of the tissue can be used without damaging the tissue (Andersson & Larsson, 1961), the use of d-Amphetamine sulfate will be far more suitable in the present context. The drug itself can produce certain side-effects such as an increase in the frequency of sniffing and gnawing behaviors but these are generally seen only with dosages much higher than those which will be used in this experiment (Randrup & Munkvad, 1967).

Amphetamine has been reported to exert a suppressive effect on water intake following a 23 hour deprivation period (Falk & Burnidge, 1970; Mogenson, 1968). Cone and Golden (1973) however, have demonstrated that the effect of amphetamine on post-deprivation water intake is not independent of the subject's circadian phase at the time of injection. Testing 24 hour water deprived 65 and 125 day old albino rats at three times of day, these researchers reported that a 1 mg/kg dosage of amphetamine produced a significant increase in post-deprivation water intake when injected during the third hour of the light

period. The same dosage had no effect when injected during the last two hours of the light period and significantly reduced post-deprivation water intake when injected at the midpoint of the dark period. The effects of the amphetamine appeared to act in a direction opposite to the normal pattern of drinking. When injected during the normally quiescent early light period, increased water intake was observed. The same general relation was observed for the other two times of day. In addition to water intake, the effects of amphetamine on locomotor activity levels have also been reported to interact with circadian cycle phase for rats (A. Cone, 1970) and for canaries (Wahlstrom & Widerlov, 1968).

The findings reported by Cone and Golden (1973) conform to the earlier hypothesis, based upon the work of Hutchinson and Renfrew (1967), of differential sensitivity to cellular dehydration as a function of adrenergic stimulation. Cone and Golden (1973) did not, however, examine the effects of an interaction of level of deprivation with adrenergic stimulation on water intake. Such an examination is necessary in order to make meaningful statements about the adrenergic control of "free-running" water intake.

Hutchinson and Renfrew's (1967) data would predict that a reduction in the length of the pre-test water

deprivation period used by Cone and Golden (1973) would result in an enhancement of the amphetamine effect. Since the proposed adrenergic regulatory mechanism is sensitive primarily to cellular dehydration (Blass, 1968), a shortened deprivation period would come much closer to simulating the conditions under which the mechanism would normally function.

Based on the work of Cone and Golden (1971, 1972), it is also more desirable to use adult hooded rats for subjects because of the stability of their "free-running" intake patterns. This stability will permit testing to be conducted at two rather than three times of day. Furthermore, in addition to the licking measure used by Cone and Golden (1973), a measure of latency to begin drinking is also needed. If the increase in water intake reported by Cone and Golden (1973) was being produced by the amphetamine, then their subjects should have begun drinking within the first hour following injection. At this point, the brain concentration of amphetamine would be approximately 87 percent of the injected dosage (Valzelli, Dolfini, Tansella & Garattini, 1968), with the half-life of the drug being approximately 75 minutes (Groppetti & Costa, 1969). Their data do not rule out the possibility that the increased intake reported for the early light period test sessions was due to a

depressive effect of the drug extending the deprivation period rather than to a direct facilitatory action of the drug on a drinking mechanism. A latency measure would permit an examination of this possibility.

The present study is designed as both a refinement and an extension of the original procedure used by Cone and Golden (1973). The results are expected to confirm these earlier findings as well as to demonstrate an increased susceptibility to adrenergic control as a result of a reduction in the duration of the deprivation period.

METHOD

Subjects

Two groups of seven male Long-Evans hooded rats, 138-168 days old (mean weight = 424g.), obtained from Rockland Farms, served as subjects. Although twelve subjects were originally assigned to each group, four of the subjects in the second group did not live to begin the experiment and one did not survive until its conclusion. As a result, five subjects were randomly dropped from the first group to balance the groups for size. The subjects were individually housed under an LD 12:12 lighting schedule (lights on at 0600 hours EDST and off at 1800 hours EDST) from 45 days of age until the conclusion of the experiment.

Apparatus

The subjects were tested in 18 x 18 x 25 cm. metal cages with 1/2" hardware cloth floors, clear 1/4" Plexiglas fronts, and 1/8" translucent plastic tops. Water in each test cage was available from a 250 ml. water bottle with a metal drinking spout. The bottle was mounted so that the 4 mm. opening in the spout was flush with the outside of a 1.8 cm. hole in the cage front. The spout and floor were wired to a transistorized drinkometer circuit modified from a design by Zucker (1969). This circuit permitted individual licks to be recorded by electromechanical counters located outside the test

chamber. In addition to the counters, the drinkometer circuits activated individual pens on an Esterline-Angus 20 channel event recorder in order to provide a measure of latency to begin drinking.

The test cages were separated by partitions, two to a shelf, on two shelves inside a specially constructed chamber. Illumination was provided by a 15 w. incandescent bulb mounted 50 cm. above the center of each cage. Light-dark periods, in accord with the subjects' home environment, were maintained in the test chamber throughout the experiment. Ventilation and masking noise were provided by an exhaust fan mounted on each shelf and centered between the two cages. Ambient temperature in both the colony and the test chamber was maintained at 78 $\pm$ 2° F. A dim red light source provided work light for the dark period test sessions. Purina Lab Chow was available ad libitum in both the home and test cages.

Procedure

The two groups of subjects were randomly assigned to begin the experiment under either the 24 hour water deprivation condition (24-1) or the one hour water deprivation condition (1-24). Each group was then randomly divided into squads of four subjects each. In order to facilitate testing, one group completed the procedure before the other group began. Before beginning the

experimental treatments, each group was deprived of water for 24 hours and then permitted to spend twelve hours in the test cages with water available. This served to adapt the subjects to the apparatus. All subjects made licking responses during the adaptation sessions. The experimental deprivation schedules were instituted following adaptation. The subjects assigned to the 24 hour deprivation condition were placed on water deprivation at 12 hour intervals beginning with the first squad at 2100 hours EDST. The subjects being tested under the one hour deprivation condition were placed on deprivation one hour before a test session was scheduled to begin. Light period testing began at 0900 hours EDST. Dark period testing began at 2100 hours EDST.

Twenty minutes prior to the beginning of a test session, the appropriate squad was removed from its home cages, weighed and given 1 ml/kg intraperitoneal injections of either isotonic saline (control), .4, .8, or 1.2 mg/ml dosages of d-Amphetamine sulfate (Sigma Chemical Co.) in an isotonic saline vehicle. Subjects were injected in the same order throughout the experiment with drug dosages randomized so that no subject received the same dosage twice in succession. Immediately following injection, each subject was placed into its test cage with the water spout turned away from the cage opening. Each test cage contained

four pre-weighed 1" (approx.) pellets of Purina Lab Chow. Ten minutes after the last injection, the water spouts were turned to the cage openings. The distance of the spout tip from the cage front was kept constant by guaging it with an index card. The subjects were permitted access to the water for a period of two hours, during which licks were recorded in one hour blocks. At the end of the test session, the subjects were removed from the test cages and returned to their home cages with water available until the beginning of the next deprivation period. Ten hours were allowed to elapse between the end of a test session and the beginning of the next deprivation period for the 24 hour deprivation condition. Thirty three hours were allowed to elapse between the end of a test session and the beginning of the next deprivation period for the one hour deprivation condition. In this fashion, squads were tested every 36 hours and alternated between the light period and the dark period on successive test sessions. At the end of each test session, food remaining in the cage as well as particles of food from the refuse paper under each cage was collected and weighed.

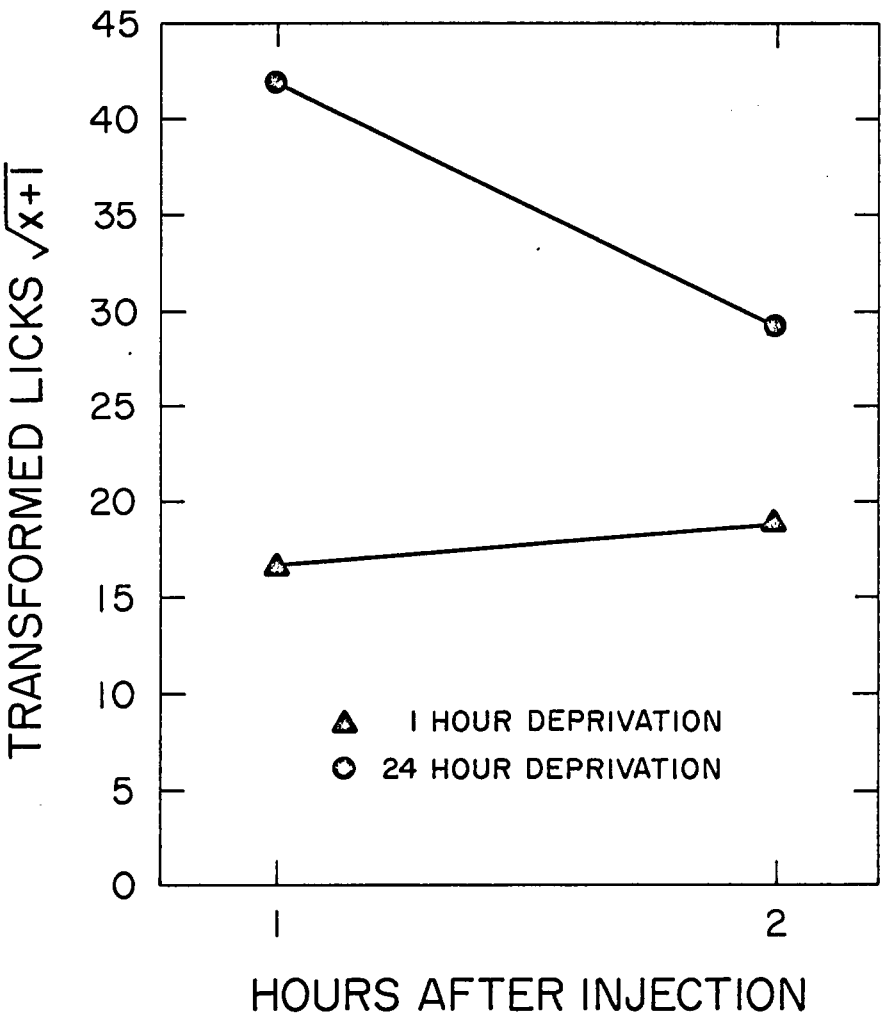
The test sessions were continued until all of the subjects received all of the drug dosages at each time of day. At this time, the deprivation conditions were reversed and the procedure repeated.

RESULTS

The lick data were transformed to the metameter $X' = \sqrt{X+1}$, as recommended by Edwards (1968) for data whose underlying distribution may be Poisson. The transformed data were then cast as a five factor mixed design Analysis of Variance with independent groups under each of the two deprivation Orders and repeated measures on the remaining four variables: Deprivation (2 levels); Circadian Phase (2 levels); Drug (4 levels); Hours after injection (2 levels).

The Analysis of Variance (Table 1) indicated that the two deprivation Order groups differed ($F=8.416$, 1/12 d.f., $p<.025$) with Group 1-24 consuming less water overall than Group 24-1. The two Deprivation period lengths produced a significant source of variability ($F=262.439$, 1/12 d.f., $p<.01$). As might be expected, 24 hours of water deprivation produced significantly more intake than one hour of water deprivation. This effect, however, was not independent of the two Hours after injection ($F=174.736$, 1/12 d.f., $p<.01$). Under the 24 hour deprivation condition, subjects showed a decrease in intake during the second hour of the test session. Under the one hour deprivation condition, no such decrease occurred (Figure 1). The Circadian Phase at the time of injection produced a significant source

Figure 1. Transformed licks as a function of the two hours after injection for each of the two deprivation conditions.

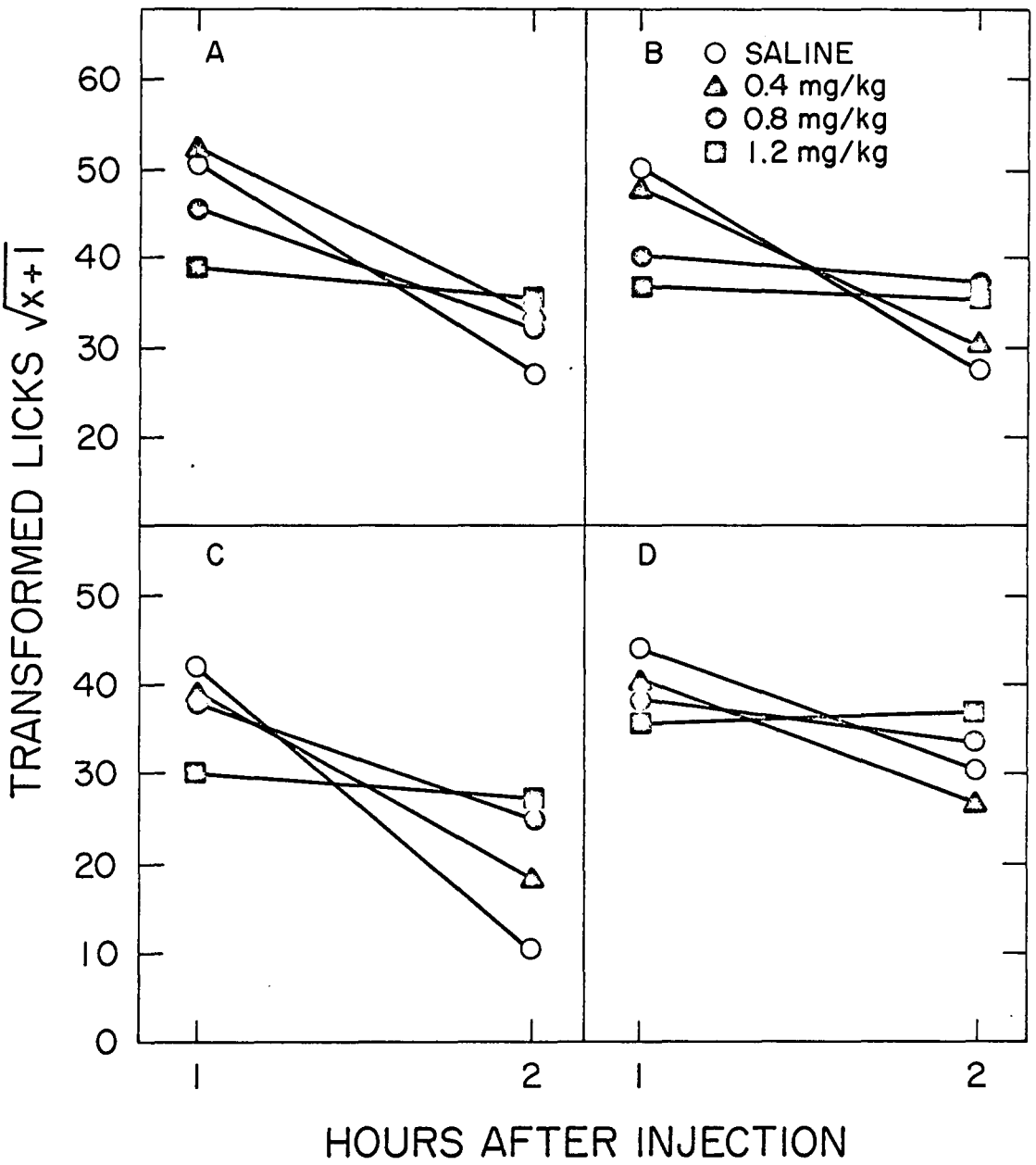

45
40
35
30
25
20
15
10
5
0
TRANSFORMED LICKS $\sqrt{x+1}$
1 HOUR DEPRIVATION
24 HOUR DEPRIVATION
1
2
HOURS AFTER INJECTION

of variability ($F=39.774$, 1/12 d.f., $p<.01$) but this effect was neither independent of the two deprivation Orders ($F=10.007$, 1/12 d.f., $p<.01$) nor of the two Hours after injection ($F=25.949$, 1/12 d.f., $p<.01$). The effects of the four Drug dosages were not independent of either deprivation Order ($F=4.013$, 3/36 d.f., $p<.025$) or of the two Hours after injection ($F=20.685$, 3/36 d.f., $p<.01$). In addition, the fourth order interaction of Order X Deprivation X Circadian Phase X Drug X Hours was significant ($F=3.105$, 3/36 d.f., $p<.05$), indicating that none of the variables could be considered to be truly independent of the others.

A series of one-way Analyses of Variance were performed to compare the drug effects under all other treatment combinations. In order to make these comparisons as conservative as possible, a grand pooled error term (93.536, 384 d.f.) was derived from the original analysis and used for each subsequent test.

Under the 24 hour deprivation condition, the data from both groups of subjects closely approximated what would be predicted on the basis of most of the available amphetamine literature. At both the AM and PM test sessions, increasing dosages of amphetamine decreased water intake during the first hour after injection. For Group 24-1 (Figures 2A and 2B), the 1.2 mg/kg dosage

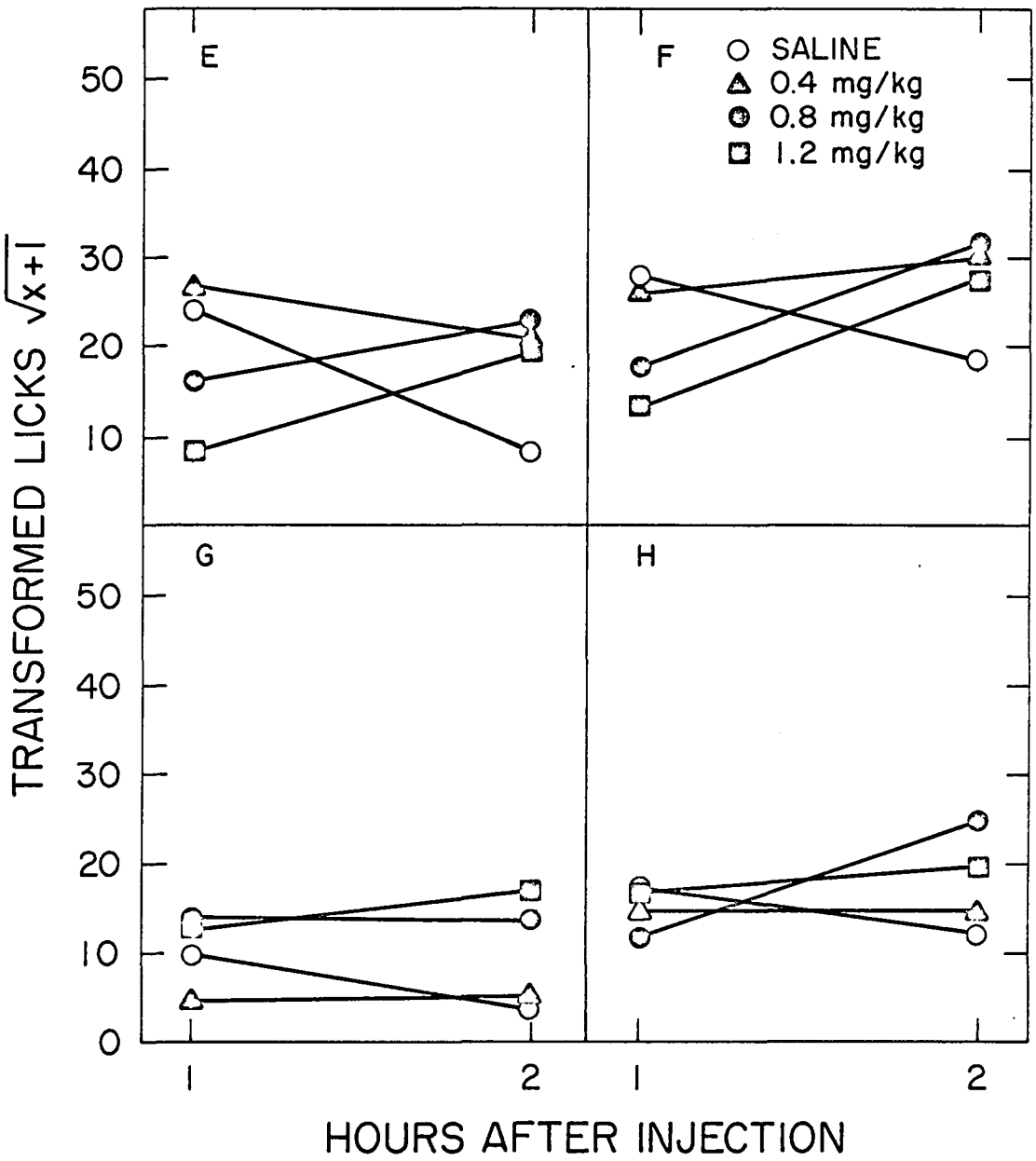
Figure 2. Transformed licks under the 24 hour water deprivation condition for each hour after injection. (A) Group 24-1 AM tests. (B) Group 24-1 PM tests. (C) Group 1-24 AM tests. (D) Group 1-24 PM tests.


A
B
C
D
SALINE
0.4 mg/kg
0.8 mg/kg
1.2 mg/kg
TRANSFORMED LICKS $\sqrt{x+1}$
HOURS AFTER INJECTION
60
50
40
30
20
10
0
1
2

produced a significant reduction in water intake over the control ($p<.05$) and .4 mg/kg ($p<.05$) drug dosages at both test times. For Group 1-24 (Figure 2C), the 1.2 mg/kg dosage also produced a significant reduction in water intake over the control dosage ($p<.05$) but not over the .4 mg/kg dosage. For this group, however, a significant reduction was found only during the AM test session, although the same order is maintained among the drug dosages in the PM test sessions (Figure 2D). During the second hour of the 24 hour deprivation condition, intake for the control and .4 mg/kg drug dosages declined to below the intake for the .8 and 1.2 mg/kg dosages.

The effects of the amphetamine were not nearly so clear cut under the one hour deprivation condition. For the AM tests on Group 24-1 (Figure 2E), the .4 mg/kg drug dosage produced a slight, non-significant, increase over control intake during the first hour. During the second hour, the .4 mg/kg drug dosage produced a significant ($p<.05$) increase over control intake. The same pattern was seen for this group during the PM test sessions (Figure 2F). During the first hour of the PM test session, the intake under the .4 mg/kg drug dosage was within 92% of control intake. During the second hour, the .4 mg/kg drug dosage produced intake which was significantly ($p<.05$) greater than control intake. Although this same

Figure 2 (continued). Transformed licks under the one hour water deprivation condition for each hour after injection. (E) Group 24-1 AM tests. (F) Group 24-1 PM tests. (G) Group 1-24 AM tests. (H) Group 1-24 PM tests.

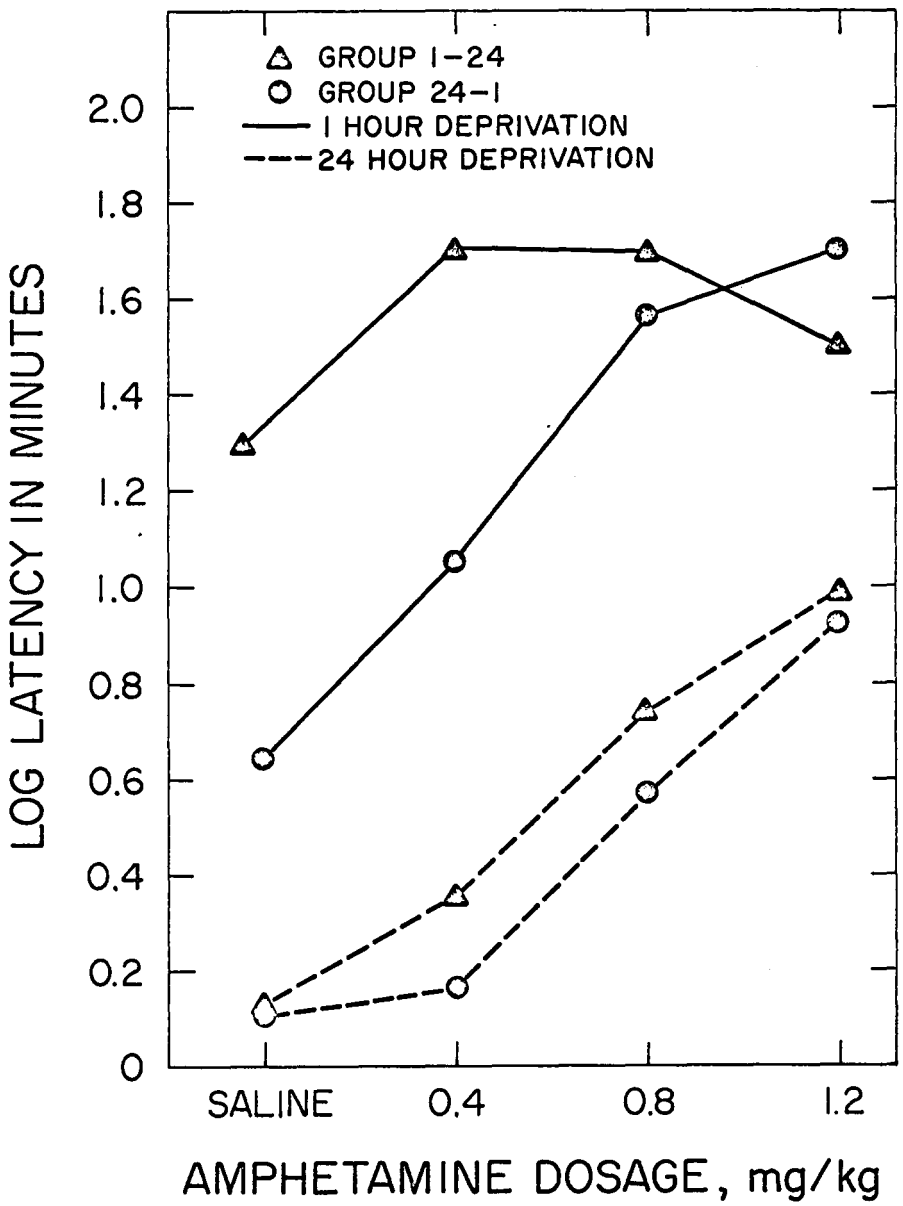

E
F
G
H
SALINE
0.4 mg/kg
0.8 mg/kg
1.2 mg/kg
TRANSFORMED LICKS √x+1
50
40
30
20
10
0
1
2
HOURS AFTER INJECTION

type of effect was seen with Group 1-24, the increase appeared with the .8 and 1.2 mg/kg drug dosages rather than with the .4 mg/kg dosage (Figures 2G and 2H).

In addition to the water intake data, Analyses of Variance were performed on both the latency to begin licking and the amount of food, in grams, consumed during the test session. These Analysis of Variance designs were the same as the one used for the water intake data with the exception that the Hours after injection variable was omitted.

The latency to begin licking was measured in minutes from the presentation of the water to the point at which a minimum of a one minute burst of licking had occurred. An examination of the raw latencies indicated that a logarithmic transformation was appropriate. The analysis of these data (Table 2) indicated that the latency to begin licking was an interactive function of deprivation Order, Deprivation period length, and Drug ($F=3.143$, 3/36 d.f., $p<.05$). It was evident, however, that the Order component of the interaction was primarily due to the data from Group 1-24 under one hour of water deprivation (Figure 3). The two groups did not differ under 24 hours of water deprivation. In addition, under one hour of water deprivation, Group 24-1 was seen to differ only in magnitude and not in form from the 24 hour deprivation

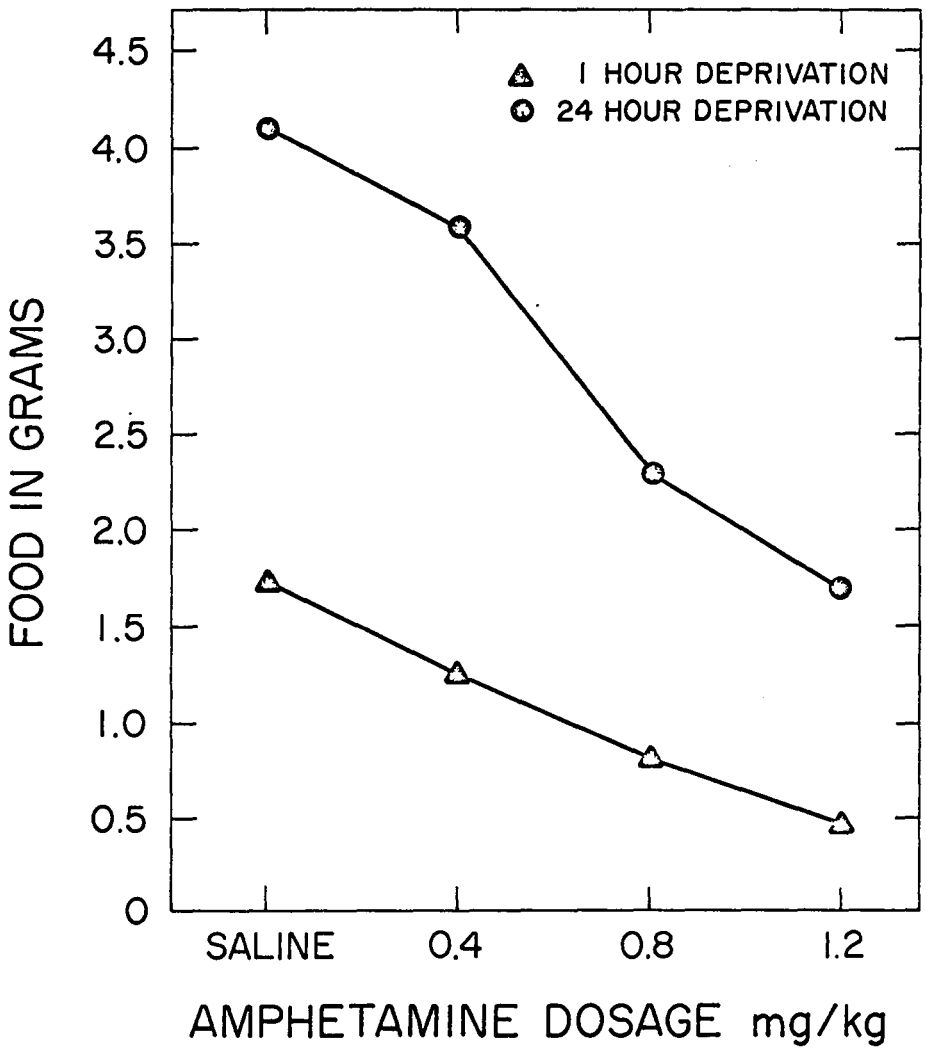
Figure 3. Log latency to begin licking as a function of water deprivation and d-Amphetamine sulfate dosage for each of the two deprivation order groups.


GROUP 1–24
GROUP 24–1
1 HOUR DEPRIVATION
24 HOUR DEPRIVATION
LOG LATENCY IN MINUTES
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
SALINE
0.4
0.8
1.2
AMPHETAMINE DOSAGE, mg/kg

condition.

Analysis of Variance of the amount of food consumed during the test sessions (Table 3) indicated that Circadian Phase produced a significant main effect ($F=15.674$, 1/12 d.f., $p<.01$) with more food being consumed during the PM test sessions. The effects of the amphetamine on food intake were not independent of the deprivation Order ($F=3.629$, 3/36 d.f., $p<.05$) or of Deprivation period length ($F=7.213$, 3/36 d.f., $p<.01$). The effects of Order were such that Group 1-24 ate less than Group 24-1 under the control and .4 mg/kg drug dosages. The two groups did not differ at the other two Drug dosages. The effect of Deprivation period length (Figure 4) was an elevation in food intake at the control and .4 mg/kg dosages under 24 hours of water deprivation. No increases above control intake were observed at any of the drug levels. The effect of the drug was an almost linear reduction in food intake. This was in contrast with the increases observed for water intake under the one hour deprivation condition.

Figure 4. Grams of food consumed during the test session as a function of d-Amphetamine sulfate dosage for each of the two deprivation period lengths.


4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
FOOD IN GRAMS
1 HOUR DEPRIVATION
24 HOUR DEPRIVATION
SALINE
0.4
0.8
1.2
AMPHETAMINE DOSAGE mg/kg

DISCUSSION

The data provide substantial support for the postulated adrenergic system controlling "free-running" water intake. Hutchinson and Renfrew's (1967) data would predict that the result of increased adrenergic stimulation would be reduced water intake in the water deprived animal and increased water intake in the non-deprived animal. Although, in order to reduce variability, one hour water deprived animals were used instead of non-deprived, this prediction was confirmed by the data from the present study. Amphetamine increased water intake in one hour water deprived animals while reducing water intake in 24 hour water deprived animals. This was the case with both deprivation order groups although the drug dosage at which the elevation occurred differed between the two groups.

The data also provide a partial confirmation of Cone and Golden's (1973) report of a circadian phase dependent shift in the effects of amphetamine on water intake. This was most evident in the data from the one hour deprivation condition and was seen primarily in the second hour after injection. Water intake increased during the PM tests above levels seen for the same hour in the AM tests. These changes indicate an increased effectiveness of lower dosages of amphetamine during the PM test sessions

and are in the direction which would be predicted on the basis of the proposed adrenergic drinking mechanism not being independent of the circadian cycle of brain norepinephrine content reported by Scheving, et. al. (1968).

The data do not, however, directly support Cone and Golden's (1973) report of increased water intake with 1 mg/kg of amphetamine following 24 hours of water deprivation. Increases were not obtained in the present study for any of the drug dosages following a 24 hour water deprivation period. It should be noted however that methodological differences exist between Cone and Golden (1973) and the present study. It would appear that the increases following 24 hours of water deprivation may have occurred primarily during the third hour following injection when these researchers report that the effects of the amphetamine had all but disappeared. This does not argue against Cone and Golden's (1973) interpretation of their results, however, since by the third hour sufficient water would have been ingested to markedly lower dehydration and the levels of amphetamine remaining in the brain tissue would have closely approximated the dosages used in the present study (Groppetti & Costa, 1969). As a result, increases would be expected to occur during the later portion of the test session. When examined in this manner, these

earlier findings also lend tentative support to the hypothesized adrenergic drinking mechanism.

Further indication that the increased water intake reported in the present study is a result of the amphetamine can be found in the latencies to begin drinking. If the increase were due to the amphetamine extending the deprivation period, followed by a compensatory rebound in water intake, then differences would be expected to occur between latencies under the control condition and under the level of the drug that was associated with the increase. This was not the case in the present study. Following the one hour deprivation period, the differences in latency between control and the drug dosages which produced increased intake (.4 mg/kg for Group 24-1 and .8 mg/kg for Group 1-24) did not differ to an extent that would account for the differences in intake. It is, therefore, reasonable to assume that the increased water intake was not artifactual but rather was produced by the amphetamine in a more direct fashion.

Oatley (1967b) has proposed that the rat's nocturnal pattern of water intake does not exist independently of food intake. It is critical for the proposed adrenergic drinking mechanism that it be demonstrated to be independent of food intake. Only in this fashion can

conclusions be drawn concerning the regulation of "free-running" water intake. In the present study, the amount of food consumed under the various levels of amphetamine showed a consistent decrease with increasing dosages of the drug. The only increase in food consumption was produced by the 24 hour water deprivation period. No increases in food intake were observed which might have been responsible for the increased water intake. At the lower dosages, the amphetamine appeared to act independently on food and water intake producing changes in opposite directions from control levels. Once again, this lends support to the proposed drinking mechanism.

Although the two deprivation order groups did differ in their responses to the drug under the one hour deprivation condition, the high pre-experimental mortality rate in Group 1-24 as well as this group's rather broad weight range (309-532g.) as compared to Group 24-1 (400-457g.) make precise statements difficult concerning the source of this difference. It should be noted, however, that both groups responded similarly to the amphetamine although at different dosages.

As can be seen, the results of the present experiment indicate the presence of an adrenergic system for the control of "free-running" water intake. The ability to increase water intake by increasing adrenergic stimulation

is neither independent of level of deprivation nor of circadian fluctuations in endogenous adrenergic transmitters. Little can be said, however, concerning neural substrates which may be involved in the process, since actual manipulation of specific neural loci was not conducted. The possible role of the pre-optic area of the hypothalamus cannot be clarified by the present experiment. However, the strong similarity between the results obtained here and those obtained by Hutchinson and Renfrew (1967) warrant further investigation of the role of adrenergic stimulation of the pre-optic area in the maintainance of fluid balance. If the present formulation is correct then damage to the pre-optic area should disrupt the circadian pattern of water intake. It should also alter the response to amphetamine induced stimulation. These questions will require additional research before they can be adequately answered.

REFERENCES

Andersson, B., Gale, C., Hokfelt, B. and Larsson, B. Acute and chronic effects of preoptic lesions. Acta Physiologica Scandinavica, 1965, 65, 45-60.

Andersson, B. and Larsson, B. Influence of local temperature changes in the preoptic area and rostral hypothalamus on the regulation of food and water intake. Acta Physiologica Scandinavica, 1961, 52, 75-89.

Andersson, B. and McCann, S. Drinking, antidiuresis and milk ejection from electrical stimulation within the hypothalamus of the goat. Acta Physiologica Scandinavica, 1955, 35, 191-201.

Blass, E. Separation of cellular from extracellular controls of drinking in rats by frontal brain damage. Science, 1968, 162, 1501-1503.

Blass, E. and Chapman, H. An evaluation of the contribution of cholinergic mechanisms to thirst. Physiology and Behavior, 1971, 7, 679-686.

Block, M. and Fisher, A. Anticholinergic central blockade of salt-aroused and deprivation-induced drinking. Physiology and Behavior, 1970, 5, 525-527.

Bolles, R. Consummatory behavior in rats maintained a-periodically. Journal of Comparative and Physiological Psychology, 1965, 60, 239-243.

Booth, D. Modulation of the feeding response to peripheral insulin, 2-deoxyglucose or 3-O-methyl glucose injection. Physiology and Behavior, 1972, 8, 1069-1076.

Carr, L. and Moore, K. Norepinephrine: Release from brain by d-amphetamine in vivo. Science, 1969, 164, 322-323.

Cone, A. Effects of amphetamine and time of day on tilt cage activity in the albino rat. Paper presented at the meeting of the Southern Society for Philosophy and Psychology, Durham, March 1970.

Cone, A. and Cone, D. Cage activity in the California antelope ground squirrel. Psychonomic Science, 1969(a), 17, 301-302.

Cone, A. and Golden, A. Effects of age and time of day of injection on amphetamine-induced adypsia in albino rats. Psychological Record, 1973, 23, 185-189.

Cone, D. Effects of illumination and circadian cycle phase on cage activity in cottontail rabbits. Paper presented at the meeting of the Southern Society for Philosophy and Psychology, Durham, March 1970.

Cone, D. and Cone, A. Cage activity in the Virginia opossum. Psychonomic Science, 1968, 10, 259-260.

Cone, D. and Cone, A. Cage activity in recently weaned albino rats, guinea pigs and wild rabbits. Paper presented at the meeting of the Virginia Academy of Science, Fredericksburg, May 1969(b).

Cone, D., Cone, A., Golden, A. and Sanders, S. Differential lick rates in opossum: A challenge to the invariance hypothesis. Psychological Record, 1973, 23, 343-348.

Cone, D. and Golden, A. Ontogeny of circadian drinking patterns of the albino rat. Paper presented at the meeting of the Southern Society for Philosophy and Psychology, Athens, April 1971.

Cone, D. and Golden, A. Ontogeny of circadian drinking patterns in Long-Evans rats. Paper presented at the meeting of the Southeastern Psychological Association, Atlanta, April 1972.

Edwards, A. Experimental Design in Psychological Research. New York: Holt, Rinehart and Winston, Inc., 1968.

Evans, H. Rats' activity: Influence of light-dark cycle, food presentation and deprivation. Physiology and Behavior, 1971, 7, 455-460.

Falk, J. and Burnidge, G. Drug antagonism and water intake. Physiology and Behavior, 1970, 5, 193-198.

Fisher, A. and Courey, J. Chemical tracing of neural pathways mediating the thirst drive. In: Thirst, edited by M. J. Wayner. Oxford: Pergamon Press, 1964.

Fitzsimons, J. Normal drinking in rats. Journal of Physiology, 1957, 138, 39.

Groppetti, A. and Costa, E. Factors affecting the rate of disappearance of amphetamine in rats. International Journal of Neuropharmacology, 1969, 8, 209-215.

Hobbs, S., Miller, C., Bunnell, B. and Peacock, L. General activity in hamsters with septal lesions. Physiology and Behavior, 1972, 9, 349-352.

Holdstock, T., Blesovsky, L. and Verschoor, G. Effects of home cage lighting on drinking behavior of rats with septal lesions. Physiology and Behavior, 1972, 8, 847-852.

Hsaio, S. and Trankina, F. Thirst-hunger interaction: Effects of body-fluid restoration on food and water intake in water-deprived rats. Journal of Comparative and Physiological Psychology, 1969, 69, 448-453.

Hutchinson, R. and Renfrew, J. Modifications of eating and drinking: Interactions between chemical agent, deprivation state, and site of stimulation. Journal of Comparative and Physiological Psychology, 1967, 63, 408-416.

Jouvet, M. The states of sleep. Scientific American, 1967, 216, 62-72.

Krikstone, B. and Levitt, R. Interactions between water deprivation and chemical brain stimulation. Journal of Comparative and Physiological Psychology, 1970, 71, 334-339.

Levitt, R. and Fisher, A. Anticholinergic blockade of centrally induced thirst. Science, 1966, 154, 520-522.

Lord, R. Seasonal changes in the activity of penned cottontail rabbits. Animal Behavior, 1964, 12, 38-41.

Mogenson, G. Effects of amphetamine on self-stimulation and induced drinking. Physiology and Behavior, 1968, 3, 133-136.

Oatley, K. Diurnal influences on post deprivational drinking in rats. Journal of Comparative and Physiological Psychology, 1967(a), 64, 183-185.

Oatley, K. Drinking in response to salt injections at different times of day. Psychonomic Science, 1967(b), 9, 439-440.

Quay, W. Effects of cutting nervi conarii and tentorium cerebelli on pineal composition and activity shifting following reversal of photoperiod. Physiology and Behavior, 1971, 6, 681-688.

Randrup, A. and Munkvad, I. Stereotyped activities produced by amphetamine in several animal species and man. Psychopharmacologia, 1967, 11, 300-310.

Scheving, L., Harrison, W., Gordon, P. and Pauly, J. Daily fluctuations (circadian and ultradian) in biogenic amines of the rat brain. American Journal of Physiology, 1968, 214, 166-173.

Siegel, P. and Stuckey, H. The diurnal course of water and food intake in the normal mature rat. Journal of Comparative and Physiological Psychology, 1947, 40, 365-370.

Stein, L. and Wise, D. Release of norepinephrine from hypothalamus and amygdala by rewarding medial forebrain bundle stimulation and amphetamine. Journal of Comparative and Physiological Psychology, 1969, 67, 189-198.

Valzelli, L., Dolfini, E., Tansella, M and Garattini, S. Activity of centrally acting drugs on amphetamine metabolism. Journal of Pharmacy and Pharmacology, 1968, 20, 595-599.

Wahlstrom, G. and Widerlov, E. The effects of (±)-amphetamine sulfate on the self-selected circadian rhythm of activity and rest in the canary. British Journal of Pharmacology, 1968, 34, 218.

Young, P. and Richey, H. Diurnal drinking patterns in the rat. Journal of Comparative and Physiological Psychology, 1952, 45, 80-89.

Zucker, I. Light-dark rhythms in rat eating and drinking behavior. Physiology and Behavior, 1971, 6, 115-126.

Zucker, M. Electronic Circuits for the Behavioral and Biomedical Sciences. San Francisco: W. H. Freeman, 1969.

APPENDIX A

Analysis of Variance tables for the three measures: transformed licks; log latency to begin licking; and grams of food consumed during the test session.

TABLE 1

Analysis of Variance for Transformed Lick Data

Source	df	MS	F
Order (A)	1	6231.110	8.416 **
error	12	740.377	
Deprivation (B)	1	36595.600	262.439 ***
A x B	1	63.473	.455
error	12	139.444	
Circadian Phase (C)	1	2243.330	39.774 ***
A x C	1	564.408	10.007 ***
error	12	56.402	
B x C	1	160.793	2.531
A x B x C	1	228.375	3.594
error	12	63.535	
Drug (D)	3	155.937	1.226
A x D	3	510.449	4.013 **
error	36	127.200	
B x D	3	38.780	.738
A x B x D	3	122.938	2.338
error	36	52.572	
C x D	3	38.545	.411
A x C x D	3	70.306	.749
error	36	93.886	
B x C x D	3	40.019	.596
A x B x C x D	3	19.530	.291
error	36	67.147	
Hours (E)	1	3148.370	15.273 ***
A x E	1	8.941	.043
error	12	206.139	

* $p < .05$
** $p < .025$
*** $p < .01$

TABLE 1 (continued)

Analysis of Variance for Transformed Lick Data

Source	df	MS	F	
B x E	1	6119.840	174.736	***
A x B x E	1	18.446	.527	
error	12	35.023		
C x E	1	1107.640	25.949	***
A x C x E	1	2.667	.062	
error	12	42.685		
B x C x E	1	8.754	.279	
A X B x C x E	1	110.450	3.523	
error	12	31.350		
D x E	3	2056.420	20.685	***
A x D x E	3	58.382	.587	
error	36	99.416		
B x D x E	3	111.476	1.724	
A x B x D x E	3	64.530	.998	
error	36	64.645		
C x D x E	3	66.017	2.099	
A x C x D x E	3	37.529	1.193	
error	36	31.455		
B x C x D x E	3	18.109	.744	
A x B x C x D x E	3	75.537	3.105	*
error	36	24.326		

* $p < .05$
** $p < .025$
*** $p < .01$

TABLE 2

Analysis of Variance for Log Latency to Begin Licking

Source	df	MS	F	
Order (A)	1	3.8770	3.057	
error	12	1.2684		
Deprivation (B)	1	43.2166	121.410	**
A x B	1	1.2735	3.578	
error	12	.3559		
Circadian Phase (C)	1	.1184	.676	
A x C	1	.2864	1.636	
error	12	.1750		
B x C	1	.3786	2.250	
A x B x C	1	.0357	.213	
error	12	.1628		
Drug (D)	3	6.0573	20.085	**
A x D	3	.7651	2.537	
error	36	.3015		
B x D	3	.4310	2.177	
A x B x D	3	.6222	3.143	*
error	36	.1979		
C x D	3	.0288	.115	
A x C x D	3	.2156	.863	
error	36	.2499		
B x C x D	3	.0319	.195	
A x B x C x D	3	.1855	1.134	
error	36	.1636		

* $p < .05$
** $p < .01$

TABLE 3

Analysis of Variance for Food Eaten During Test Session

Source	df	MS	F
Order (A)	1	50.9200	6.546 *
error	12	7.7789	
Deprivation (B)	1	188.7060	128.765 ***
A x B	1	.9836	.671
error	12	1.4655	
Circadian Phase (C)	1	27.7204	15.674 ***
A x C	1	.2865	.162
error	12	1.7686	
B x C	1	1.5169	1.710
A x B x C	1	.8690	.980
error	12	.8870	
Drug (D)	3	38.5424	22.775 ***
A x D	3	6.1410	3.629 **
error	36	1.6923	
B x D	3	5.2314	7.213 ***
A x B x D	3	1.8483	2.548
error	36	.7253	
C x D	3	.9589	1.228
A x C x D	3	1.4350	1.838
error	36	.7808	
B x C x D	3	.6292	.726
A x B x C x D	3	.0475	.055
error	36	.8662	

* $p < .05$
** $p < .025$
*** $p < .01$

The two page vita has been removed from the scanned document. Page 1 of 2

The two page vita has been removed from the scanned document. Page 2 of 2

CIRCADIAN PHASE DEPENDENT EFFECTS OF D-AMPHETAMINE SULFATE AND LEVEL OF DEPRIVATION ON WATER INTAKE IN HOODED RATS

by

Anthony John Golden

(ABSTRACT)

Two groups of seven male hooded rats were tested following one and 24 hours of water deprivation under each of four levels of d-Amphetamine sulfate (control, .4, .8, 1.2 mg/kg) at 0900 and 2100 hours EDST. Licks upon a water spout were recorded for two hours after the drug injection. In addition, measures of the latency to begin licking and the amount of food consumed were also taken at each test session. All of the variables examined interacted significantly. Following 24 hours of water deprivation, amphetamine reduced both food and water intake at both times of day. Following one hour of water deprivation, the low dosages of amphetamine produced a significant increase in water intake at both times of day. This increase did not occur for food intake. The results are discussed in terms of an adrenergic mechanism which controls "free-running" water intake in the rat independently of food intake.