THE INFLUENCE OF SUBCUTANEOUSLY INJECTED SODIUM CHLORIDE SOLUTIONS ON READINESS TO DRINK AND AMOUNT OF WATER CONSUMED BY ALBINO RATS

THESIS FOR THE DEGREE OF M. A.
MICHIGAN STATE UNIVERSITY

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1966

THE INFLUENCE OF SUBCUTANEOUSLY INJECTED SODIUM CHLORIDE SOLUTIONS ON READINESS TO DRINK AND AMOUNT OF WATER CONSUMED BY ALBINO RATS

By

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A THESIS

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

MASTER OF ARTS

Department of Psychology

1966

ACKNOWLEDGMENT

The author expresses appreciation to Dr. Glenn I. Hatton and Dr. John I. Johnson for their aid in preparation of this thesis. The author is grateful to Mrs. Thornton for typing the drafts.

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GENERAL INTRODUCTION

Essential to a complete understanding of the many processes of water regulation is knowledge of the factors leading to the initiation of drinking behavior. Many investigators have considered changes in blood plasma osmolarity to be of critical importance for the instigation of water ingestion as well as for the determination of the amount of water ingested. Using male hooded rats, Wayner (1964) found that water consumption varied directly with the concentration of saline solutions injected subcutaneously. Corbit (1965) obtained a similar relationship between amount drunk and concentration of intravenously infused saline solutions in albino rats. Also using albino rats, Hatton (1965) has observed changes in the electrical activity of the lateral hypothalamus following subcutaneous injections of hypertonic saline solutions. The latency between injection and electrical change in hydrated rats was found to be approximately five minutes. It seems reasonable that this time lapse is necessary for the occurrence of plasma changes sufficient to trigger some osmodetection mechanism. The electrophysiological changes, then, could be related to the instigation of drinking behavior in the intact, awake animal.

O'Kelly (1963) presented some essential processes involved in regulatory behavior. The processes and their definitions are;

(a) "detector" functions, whereby significant perturbations of relevant system variables can be discovered and can initiate the regulatory sequence;

(b) selective "orientation and discrimination" functions that enable the organism to respond differentially and appropriately to the environment stimulus possibilities;

(c) "correctional" functions which act in such a fashion as to restore system steady states, e.g. motor programs of sucking, chewing, swallowing, etc., in hunger or thirst; and

(d) "satiety" functions, serving to terminate the corrective motor activity.

It is possible to hypothesize that some portion of the lateral hypothalamus serves as one of the structures underlying the detector function. Supporting data for this hypothesis, in addition to the electrophysiological observations mentioned above, are available. Anderson and McCann (1955) induced drinking in goats with electrical stimulation of the lateral hypothalamus. Greer (1955) has shown that electrically stimulating the lateral hypothalamic area increases drinking in rats. After removing parts of the lateral hypothalamic area by lesioning, Montemurro and Stevenson (1957) found that dehydrated rats did not drink.

In our laboratory, a program of research is directed towards identifying the neural structures which detect changes in plasma osmolarity and, thus, participate in the

initiation of water ingestion. There are no behavioral data available which allow direct comparison with the changes in electrical activity which have been observed following subcutaneous saline injections. It is necessary to know if the onset of drinking is temporally correlated with the electrophysiological changes in the hypothalamus. The present set of experiments were planned in part to establish this temporal relationship.

These studies were also designed to provide a partial answer to the question asked by O'Kelly, page 75;

> How well does the vertebrate recognize his internal state of hydration or dehydration?

Is the rat capable not only of discriminating between dehydration and satiation, but also discriminating between different degrees of dehydration? The present experiments attempt to establish the ability of the rat to discriminate between different degrees of elevated plasma osmolarity, using readiness to drink as the index of discrimination.

Bolles (1962) has defined readiness to drink as the time lapse between access to water and the initiation of water ingestion.

Amount of water ingested in a predetermined time period is the behavioral index used as a post hoc measure of degree of dehydration. Previous research has demonstrated this to be a reliable index.

EXPERIMENT I

The variable on which hypothalamic activity and behavioral activity were compared in this experiment was the time for the change in plasma osmolarity to be detected as indicated by readiness to drink. The experiment rests on two assumptions: (a) that detection is accompanied by electrical activity in the lateral hypothalamus, and (b) that detection results in initiation of drinking behavior.

To make the data from this study comparable to electrophysiological data, subcutaneous sodium chloride injections were used to initiate drinking behavior in the rats. The literature is replete with studies employing this technique, e.g. Young, Heyer, and Rickey (1952); Wayner, Wetrus, and Blonk (1962); and Heyer (1951).

To establish a relationship between degree of dehydration and readiness to drink and amount drunk, the rats were injected with different concentrations of sodium chloride solution. The experiment, then, was a parametric study of the initiation and amount of drinking following injections of different concentrations of saline solution.

Method

Subjects

Twenty-four male albino rats, obtained from Spartan Research Animals, were housed in individual cages in a

temperature--controlled room, with constant light. The rats weighed approximately 300 ± 10 grams on arrival.

Apparatus

The drinking box with six compartments is described in the appendix. Six graduated 100 milliliter gas measuring tubes were used to hold the water and measure the amount of water consumed by the rat during the drinking session. A clock, with hundredth second divisions, was used to measure latencies. A foot pedal switch started and stopped the clock. Six 1 cubic (one per rat) centimeter syringes (24 ga.) were used for subcutaneous injections. An animal restrainer described in the appendix, was used to hold the rate while giving the salt injections.

Procedure

On the day of arrival, twenty-four rats were assigned to two treatment groups. The first group received an ascending series of injections: .87%, 4%, 8%, 16% and .87%; and the other group received a descending series of injections: .87%, 16%, 8%, 4%, and .87%.

Subjects were adapted to a 23 1/2 hour water deprivation schedule and to the drinking box. During this session, latency to drink after water was made available and amount of water drunk was recorded. A drinking response was defined as licking at the spout for three or more seconds. After all individual rats' readiness to

drink had not systematically increased for five days, the series of injections was begun.

The rats were placed in the drinking box with water available for a half hour throughout the adaptation period and the treatment period. During this period, both latency to drink and amount drunk were recorded. After the half hour of drinking on treatment days, the six animals to be tested were removed from the drinking box, placed in the restrainer, injected with lcc of salt solution subcutaneously in the back at the level of the hind legs, and placed back into the drinking box. The criterion for subcutaneous placement of the needle was free movement of the needle between the skin and flesh of the rat. Time from injection to initiation of drinking was measured, and Ss were allowed to drink for an additional 30 minutes, after which, they were removed from the drinking box, weighed, and placed back into their home cages. The amount of water consumed after injection was then recorded.

Results

The readiness to drink data from the treatment conditions is presented in a histogram for quick meaningful inspection, and as medians and ranges of latencies to drink after injections of different concentrations of saline solutions. The histogram presents the data before transformations are made in the following statistical

analyses. The latency to drink data for the six treatment conditions, (first .87% saline injections, Last .87% saline injection, 16% saline injection, 8% saline injection, and 4% saline injection), are presented in Figure 1.

The medians and ranges of latency to drink, in seconds, after injection of different concentrations of saline solutions are presented in Table 1.

The overall median latency to drink for all Ss after injections of 4%, 8%, and 16%, was 321 seconds.

The data collected from the drinking behavior during the adaptation stage of the experiment and drinking behavior after 23 1/2 hour water deprivation throughout the treatment stage of the experiment are presented in Figure 2.

A t test was used to determine if the amount drunk by the 24 rats after 23 1/2 hour deprivation and before injection of saline solutions on all treatment days was different from the amount drunk by the same 24 rats after 23 1/2 hour water deprivation and the day after injection of saline solutions on all treatment days. A t test for correlated observations was used. A two tailed t test for correlated observations was used to determine if the amount of water drunk by the 24 albino rats after injection of .87% saline solution was different from the amount of water drunk by the same 24 albino rats after injection of 4% saline solution.

TABLE 1

Medians and ranges of latency to drink, in seconds, after injection of saline solutions.

N	First .87%		4%		8%		16%		Last .87%	
	Median	Range	Median	Range	Median	Range	Median	Range	Median	Range
24	1,189.5	137-11,708	437.5	130-3,591	308	209-1,706	322	119-647	552.5	110-19,800

FIGURE 1

Latency to drink of the 24 albino rats immediately after injection of saline solutions for each saline concentration.

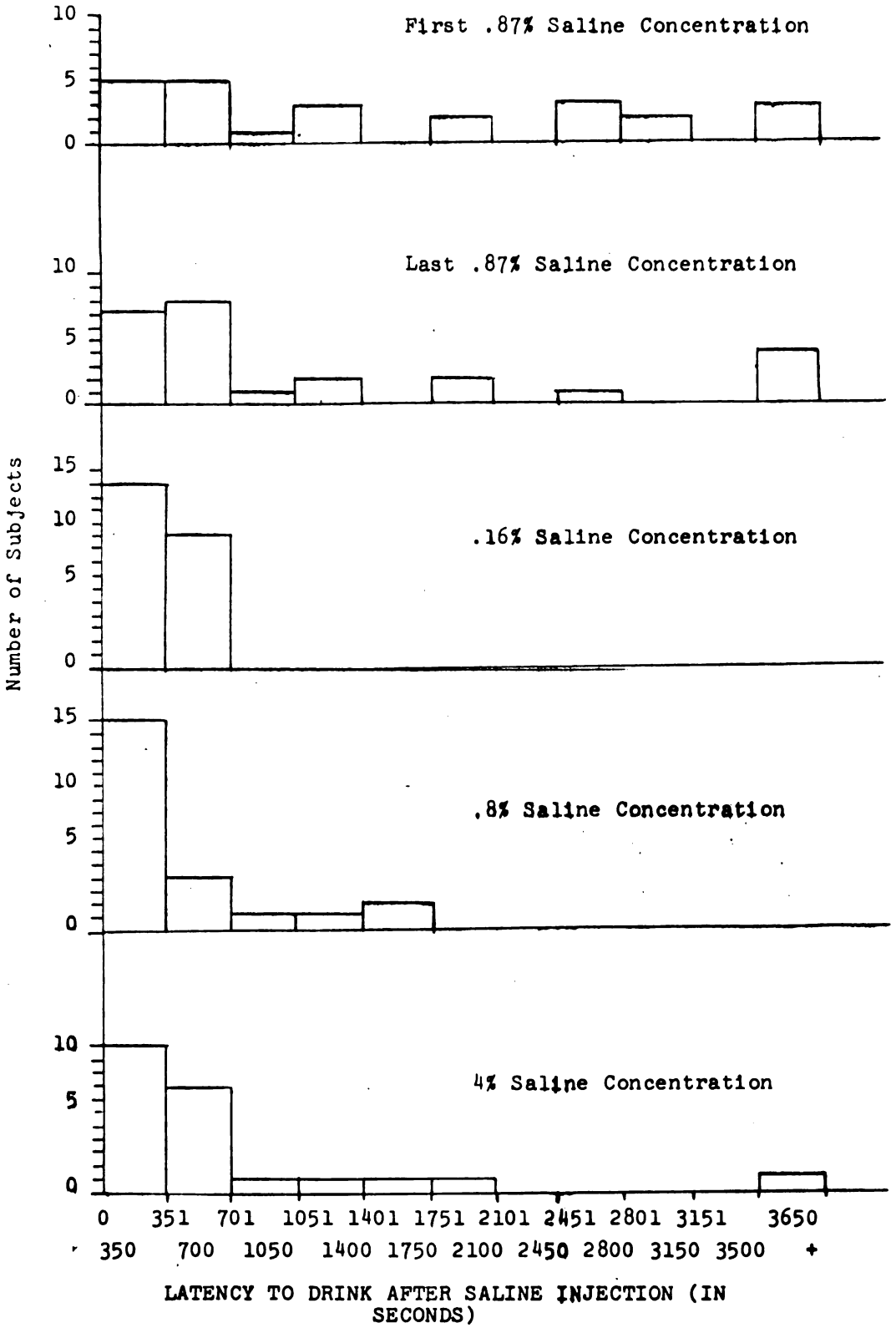
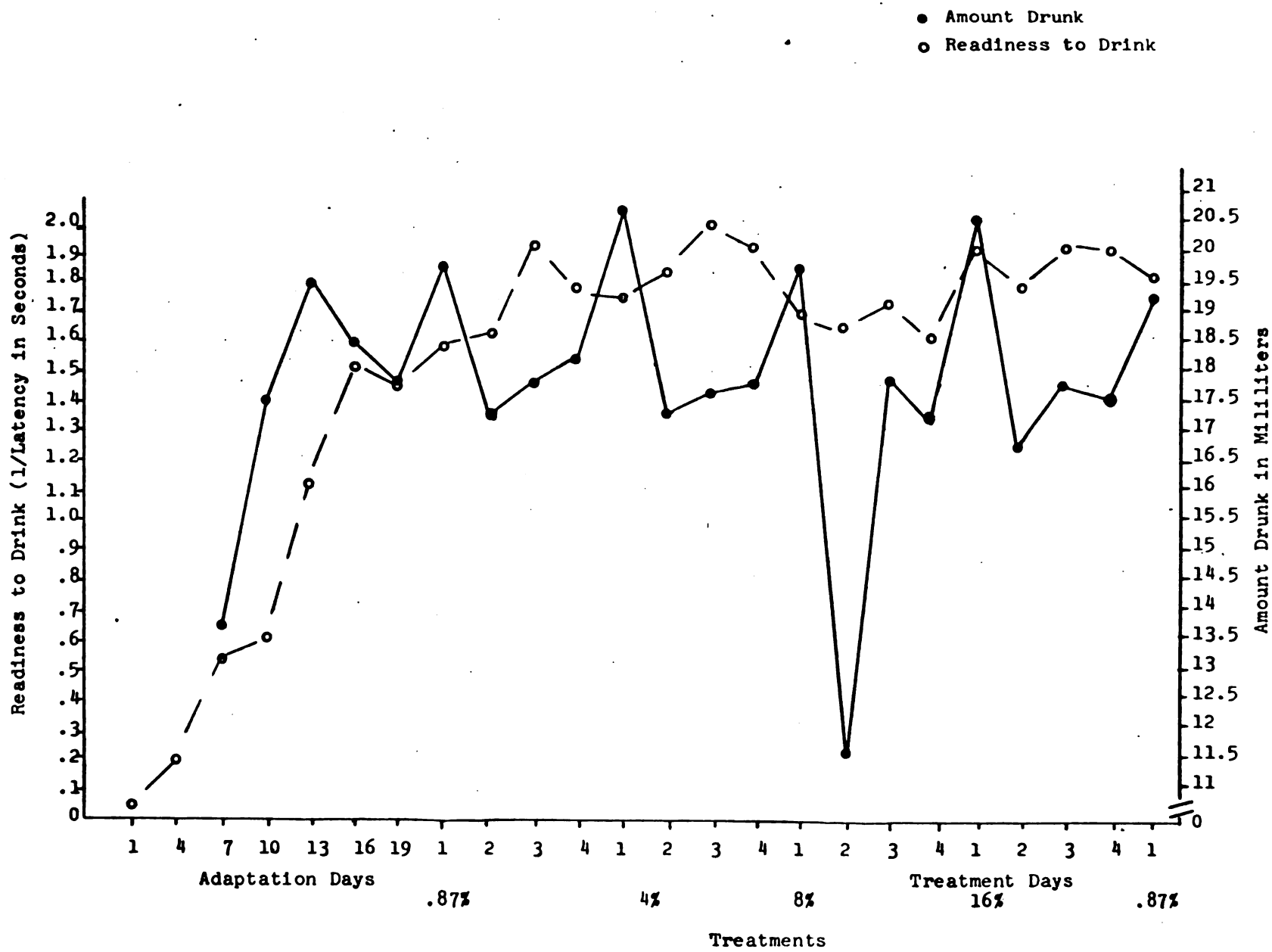

First .87% Saline Concentration
Last .87% Saline Concentration
.16% Saline Concentration
.8% Saline Concentration
4% Saline Concentration
Number of Subjects
10
5
0
15
0 351 701 1051 1401 1751 2101 2451 2801 3151 3650
350 700 1050 1400 1750 2100 2450 2800 3150 3500 +
LATENCY TO DRINK AFTER SALINE INJECTION (IN SECONDS)

FIGURE 2

Readiness to drink and amount drunk by the 24 albino rats after 23 1/2 hour water deprivation as a function of days of habituation period and treatment conditions. The ordinate on the left side of the graph presents the mean reciprocal latenices to drink. The ordinate on the right side of the graph presents the mean mililiters of water drunk. The left half of the abscissa presents the succeeding days of adaptation. The right half of the abscissa presents the day of injection of each concentration of saline solution, followed by the three days after the day of injection. The readiness to drink data and amount drunk data are after 23 1/2 hour water deprivation. The readiness to drink data and amount drunk data after injections are presented in another graph (Figure 3). Each data point is determined from 24 male albino rats.


Amount Drunk
Readiness to Drink
Readiness to Drink (1/Latency in Seconds)
Amount Drunk in Milliliters
Adaptation Days
Treatment Days
.87%
4%
8%
16%
.87%
Treatments

In Figure 2 both readiness to drink of the 24 albino rats and amount of water drunk are plotted as a function of days.

The data points on Figure 2 show that the amount of water drunk at normal daily drinking sessions following injection days is less than the amount of water drunk during the normal daily drinking session of the injection day. A t test of the difference between the mean amount drunk by 24 albino rats after 23 1/2 hour water deprivation on the treatment day and the mean amount drunk by 24 albino rats after 23 1/2 hour water deprivation on the day following the treatment day is significant. ($t = 12.13$, $df = 23$, $p < .025$).

The mean difference between mean reciprocal latency to drink after injections of 4% and .87% saline solutions is significant ($t = 1.74$, $df = 22$, $p < .05$). The means for amount drunk under these two conditions were not different. ($t = 1.35$, $df = 23$, $p < .05$).

The sharp decrease in amount drunk on the second day after 8% injection occurred when the rats had no food.

The data for amount of water drunk after injections of saline solutions are presented in units of milliliters of water consumed. The data is transformed by finding the difference between the amount of water drunk by each rat after injection of .87% saline solution and the amount of water drunk by the same rat after injection of 4%, after 8%, and after 16% saline solution. Each difference

was divided by the amount of water drunk by the rat after injection of .87% saline solution. The resultant member is called the percent change from control injection.

Using mean percent change from control of 24 rats, a two way analysis of variance was used to determine if concentration effects, order effects, or interaction effects were reliable. Duncan's test was used for individual comparisons.

In Figure 3 the water intake and readiness to drink data for the three injection conditions are plotted as percentages of physiological saline injected control on a standardized scale.

Statistical analysis of amount drunk, revealed that the difference between the means of the ascending and descending orders was not statistically significant ($F = 1.75$, $df = 1/22$, $p > .05$). The means of amount drunk for the three saline conditions were significantly different at $p < .001$ ($F = 42.29$, $df = 2/44$). The interaction of orders with concentrations was not statistically significant ($F = .49$, $df = 2/44$, $p > .05$).

Duncan's (Winer, 1962) test resulted in the following information: 4% Ss drank less than 8% Ss ($p < .05$); and 8% Ss drank less than 16% Ss ($p < .05$).

The data for readiness to drink after injection of saline solutions were transformed twice. The reciprocals of the latencies to drink after injection was found in order to justify using parametric statistical tests. The

FIGURE 3

Percentage change in amount drunk and readiness to drink of the 24 albino rats as a function of concentration of NaCl.

One graph is used to present the amount of water drunk by the 24 albino rats after injection of each saline solution and readiness to drink by the 24 rats after injection of each saline solution. To make the amount drunk and readiness to drink data comparable, the means of amount drunk (percent change from control) were divided by the standard deviation of the pooled data, and the means of readiness to drink (percent change from control) were divided by the standard deviation of the pooled data.

The ordinate presents of percent change from control injection. The abscissa presents the different concentrations of saline solutions injected.

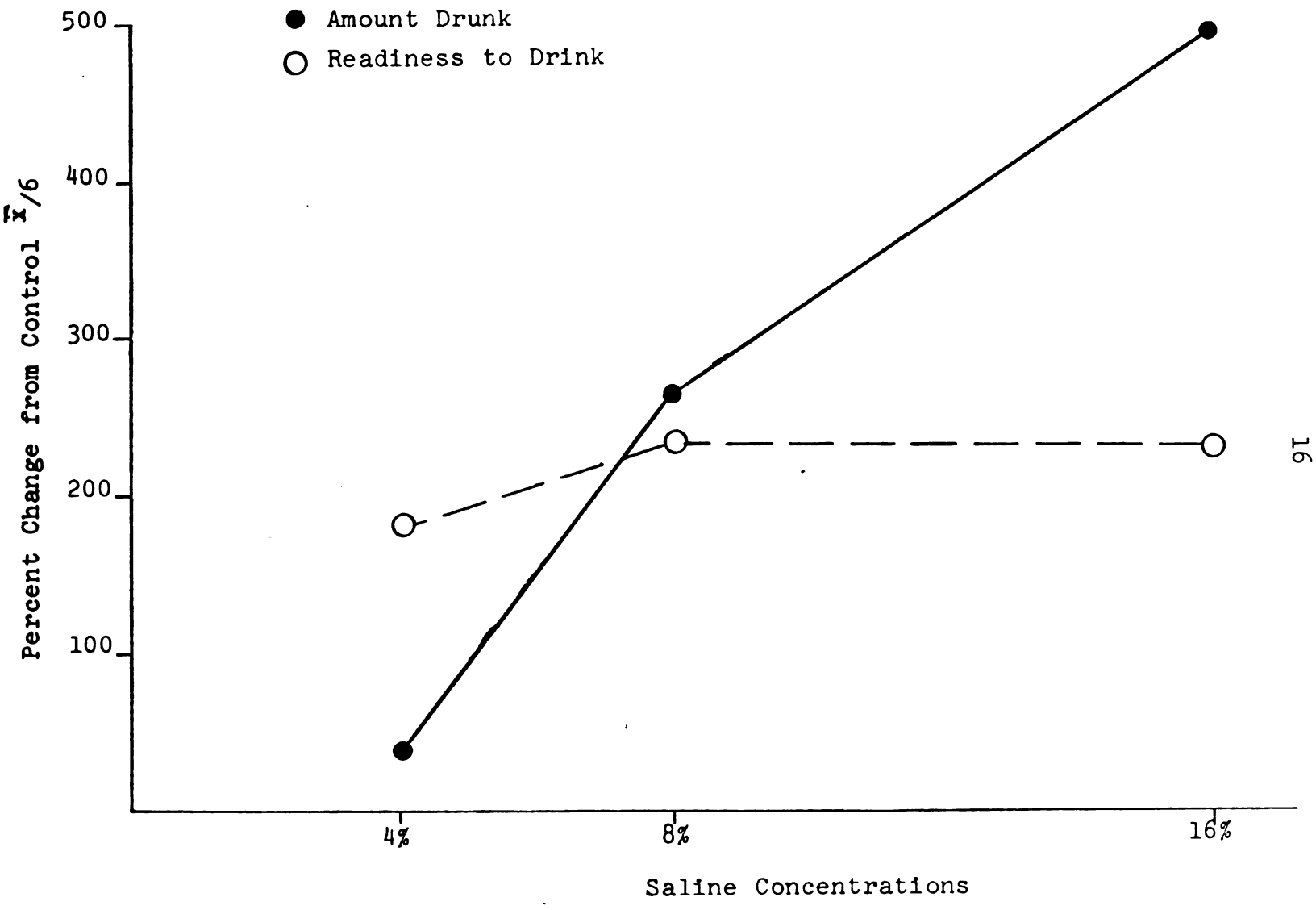

Amount Drunk
Readiness to Drink
500
400
300
200
100
Percent Change from Control $\bar{x}/6$
4%
8%
16%
Saline Concentrations

TABLE 2

Summary of Analysis of Variance
(Amount Drunk).

Between Subjects	23		
Order (O)	1	15.61	1.75
Ss within groups	22	8.92	
Within Subjects	48		
Concentration (C)	2	131.5	42.29*
O x C	2	1.54	0.49
C x Ss within gps.	44	3.11	

reciprocal latency to drink for each rat after injection of .87% saline solution was subtracted from the reciprocal latency to drink for the same rat after injection of 4%, after 8%, and after 16% saline solution. The difference for each rat was then divided by the reciprocal latency to drink for the same rat after injection of .87% saline solution. The resultant number is called the percent change from control injection. Using mean percent change from control for 24 rats, a two way analysis of variance was used to determine if concentration effects, order effects, and interaction effects were reliable. Duncan's test was used for individual comparisons.

Statistical analysis of readiness to drink revealed that the difference between ascending and descending orders was not statistically significant, ($F = 2.20$, $df = 1/22$, $p > .05$). Means of readiness to drink for the different concentrations were likewise not significantly different

(F = .68, df = 2/44, p > .05). The interaction of orders with concentrations was significant (F = 3.29, df = 2/44, p < .05).

TABLE 3

Summary of Analysis of Variance
(readiness to drink)

Source of Variation	DF	MS	F
Between Ss	23		
Order (O)	1	9.40	2.20
Ss within gps	22	4.27	
Within Ss	48		
Concentration (C)	2	.53	.68
O x C	2	2.57	3.29*
C x Ss within gps	44	.78	

Discussion

What are the possible causes of delay in time from injection to initiation of drinking behavior? Latency is determined by the access of water to the site of injection, by the ease of transport of solution between plasma and the site of injection, by the extent of dehydration of the blood, and by the change in cellular hydration level in the central neural detector, and by the threshold characteristics of that detector. Once detection has occured the effect of the change in osmolarity on behavior may be delayed by other factors, such as, knowledge

of where water is located, and effectiveness of motor acts in getting to the water.

Wayner (1964) demonstrated that serum Na in male hooded rats increased to a maximum 35 minutes after a subcutaneous injection of 3.2 miliequivalents of 15% saline solution, while drinking behavior occurred 9 minutes after injections. He also demonstrated that during the first 15 minutes, after injection of 3.2, 6.5, and 13 miliequivalents of 15% saline solution, the increase in serum Na in the rats' blood was similar. Wayner found that the normal serum Na level in the blood of the hooded rats was 145.9 miliequivalents.

The concentration of 16% (2.74 miliequivalents) saline solution in the present experiment is closest to Wayner's concentration of 3.2 miliequivalents of 15% saline solution. The median latency to drink after injection of 16% saline solution was 5 minutes 21 seconds. Factors which may account for the differences between Wayner's and the present results are adaptation to drinking box, handling procedures, lighting, temperature, humidity, strain differences, and possible site of injection.

The results in the present experiment lead to the conclusion that the amount of water drunk by albino rats in the above test situations is a montonically increasing function of concentration of subcutaneously injected saline solution. The conclusion is in agreement with other comparable experiments in the literature.

Wayner (1964) demonstrated that male hooded rats drank increasing amounts of water with increasing concentrations of subcutaneously injected saline solutions, when given 4 hours to drink after injections. Table 3, summarizes the results of the present experiment and Wayner's results.

Wayner's injections of 1 and 2 miliequivalents resulted in greater amounts of water consumed than did injections of 1.87 and 2.74 miliequivalents in the present study. The factor which presumably contributed to the consumatory difference was that Wayner allowed his rats to drink 3 1/2 hours longer than the rate in the present experiment.

Although there is no apparent relationship between the means of readiness to drink and concentration of saline solutions, the variability of readiness to drink decreases with increasing concentration. This conclusion is not in agreement with Corbit (1965). From his results of 3.66, 2.45, and 1.58 minutes to initiate drinking after intravenous injections of 5, 10, and 20% saline solutions, Corbit concluded that latency to drink was inversely related to the concentration of the injected solution. Corbit's results are in contrast to the results of the present experiment, which were 7.29, 5.13, and 5.37 minutes to drink after subcutaneous injections of 4, 8, and 16% saline solutions.

The differences in experimental procedure and injection route could explain the differences in results:

1. Corbit injected the saline solution intravenously, with 1 cc syringe at a rate of 0.2 ml/minute, which probably resulted in a faster rate of plasma concentration change than the rate of plasma concentration change after subcutaneous injection. Corbit's rats were tested in their home cage, whereas, the rats in the present experiment were tested in a drinking box outside of their home cage. The above factors could underlie the observation that Corbit's rats drank in a shorter time than the rats in the present experiment.

2. Corbit gave injections in an ascending order on successive days, whereas, in the present experiment, the latencies came from one group of ascending injections and another group of descending injections, with a three day interval between injections. Corbit's procedure of injecting increasing concentrations of saline solution on successive days might explain why he obtained decreasing latencies with increasing concentrations.

The final conclusion of the present experiment is that the two response measures are different. The responses and their measures, how long rats spend drinking, or amount drunk, and how long rats take to initiate drinking after injection of saline solution or latency to drink, were demonstrated to be different in different stages of the present experiment. In the habituation stage, total time

spent drinking and amount drunk stabilized before time to approach and initiate drinking. Water intake decreased significantly the day after a test injection, whereas, there was no apparent relationship between readiness to drink and daily drinking sessions after day of injection. Amount of water drunk increases monotonically with increases in concentration of injected saline solution, whereas, the variability of readiness to drink seems to decrease with increasing concentrations of saline solutions.

TABLE 4

Comparison of miliequivalents of NaCl injected and amount drunk in Wayner's experiment and the present experiment.

Miliequivalents of NaCl injected		Milliliters drunk	
Present Experiment	Wayner	Present Experiment	Wayner
.15		2.29	
.68		3.10	
	1		7.5
1.37		6.2	
	2		10.5
2.74		9.9	
	4		17.0
	6		26.0
	8		29.0

EXPERIMENT II

In Experiment I, readiness to drink was measured immediately after injections of different concentrations of saline solutions. The results indicated that varying the concentration of saline solution did not have a significant effect on readiness to drink. Diffusion time, and pain might have had an effect, on readiness to drink, which was differential for the various concentrations. The present experiment was designed to control for the above effects on readiness to drink. The rats were injected as in experiment I and then delayed for six hours before being given access to water. The time delay allows the saline to diffuse throughout the intra and extracellular compartments and the effects of pain to subside.

Method

Subjects

The 24 rats of Experiment I served as Ss for Experiment II, with the exception of one which died before Experiment II was begun.

Apparatus

The apparatus from Experiment I was used in Experiment II.

Procedure

The Ss were assigned to ascending and descending conditions in Experiment II such that: one-half of each group was made up of animals who had been given an ascending series of injections in the first study; the other half had received the descending series. It was after this assignment had been made that one rat, scheduled to receive a descending series, died, leaving that group with eleven Ss.

Following a period of 46 days on ad lib conditions after Experiment I, deprivation schedules were instituted as before. The procedures for habituation for this second study were similar to those of the first and the same measurements were taken.

On treatment days, Ss were removed from their home cages, allowed to drink for 1/2 hour in the drinking boxes, during which the food was removed from the home cages, injected with 0.20 cc of xylocaine and a salt solution, and returned to their living cages for a delay period of six hours. Following this delay period, Ss were again placed in the drinking boxes and allowed access to water for one hour. Treatments were administered every fourth day. Latency to drink and amount drunk were measured for each period of access to water.

Statistics and Graphs

The same statistical tests, units, and graph construction techniques were used in Experiment II, as in Experiment I.

Results

Figure 4 presents latency to drink of the 23 albino rats for each saline concentration.

In Figure 5, the readiness to drink data and the water intake data are plotted as a function of days and treatments.

In Figure 6, the water intake and readiness to drink data for the three injection conditions are plotted as percentages of change from the physiological saline control condition on a standardized scale. An analysis of variance on the water intake data showed these means to differ significantly ($F = 29.58$, $df = 2/44$, $p < .05$). Duncan's test reveals that all pairs of means differ significantly ($p < .05$).

The difference between the means of the ascending and descending orders was not statistically significant ($F = .86$, $df = 1/22$, $p > .05$). The interaction of orders with concentrations was not significant ($F = 2.63$, $df = 2/44$, $p > .05$).

Statistical analysis of the readiness to drink data revealed that the difference ascending and descending orders was not significant ($F = .09$, $df = 1/22$, $p > .05$). The interaction of orders with concentrations was not significant ($F = .18$, $df = 2/44$, $p > .05$). Means of readiness to drink for the different concentrations was significantly different ($F = 3.71$, $df = 2/44$, $p < .05$).

FIGURE 4

Latency to drink of 24 albino rats six hours after injection of saline solutions for each saline concentration.

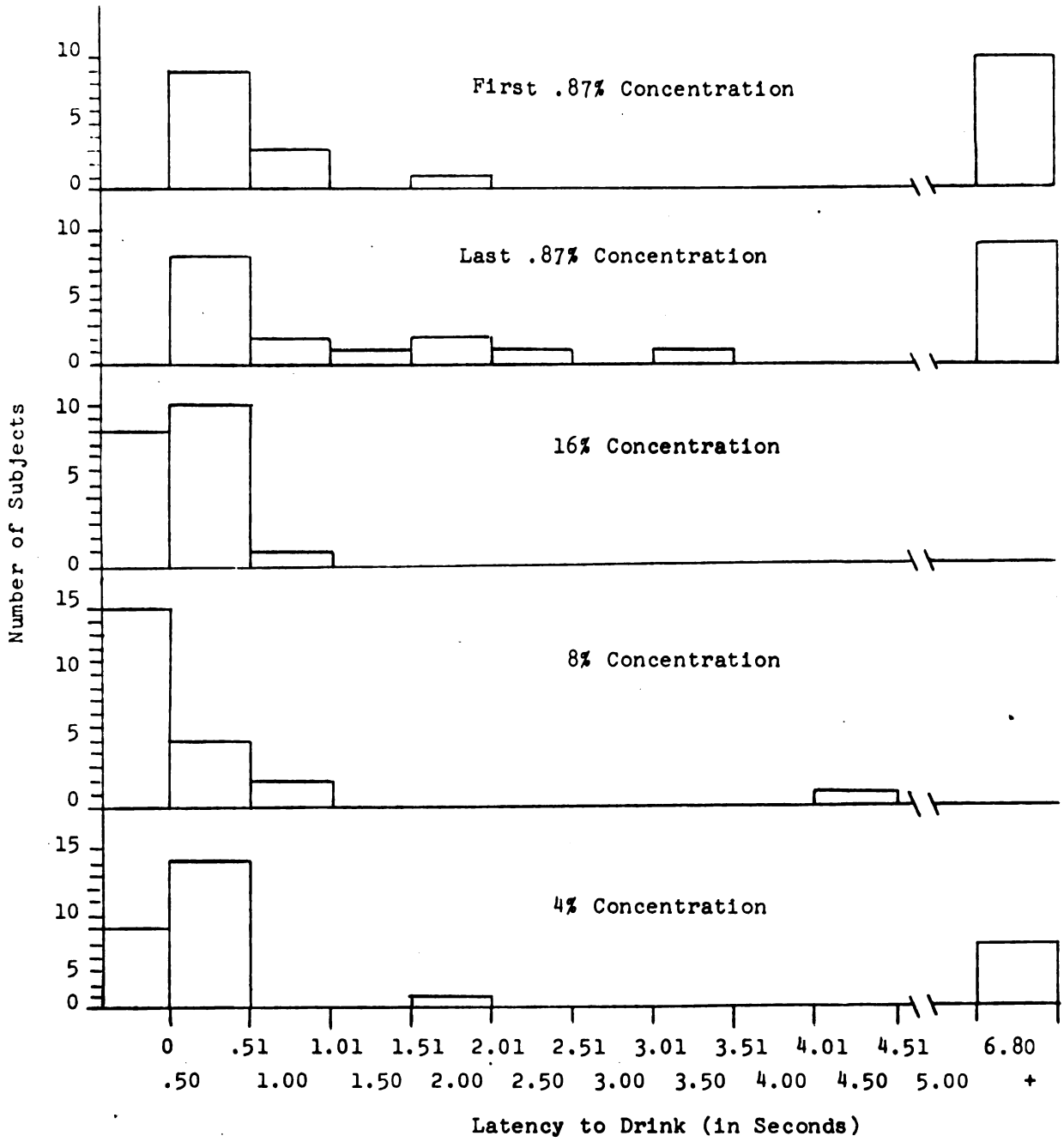

First .87% Concentration
Last .87% Concentration
16% Concentration
8% Concentration
4% Concentration
Number of Subjects
10
5
0
15
0 .51 1.01 1.51 2.01 2.51 3.01 3.51 4.01 4.51 6.80
.50 1.00 1.50 2.00 2.50 3.00 3.50 4.00 4.50 5.00 +
Latency to Drink (in Seconds)

FIGURE 5

Readiness to drink and amount drunk by 24 albino rats after 23 1/2 hour water deprivation as a function of days of habituation period and as a function of days on and after treatment conditions. (Before injection)

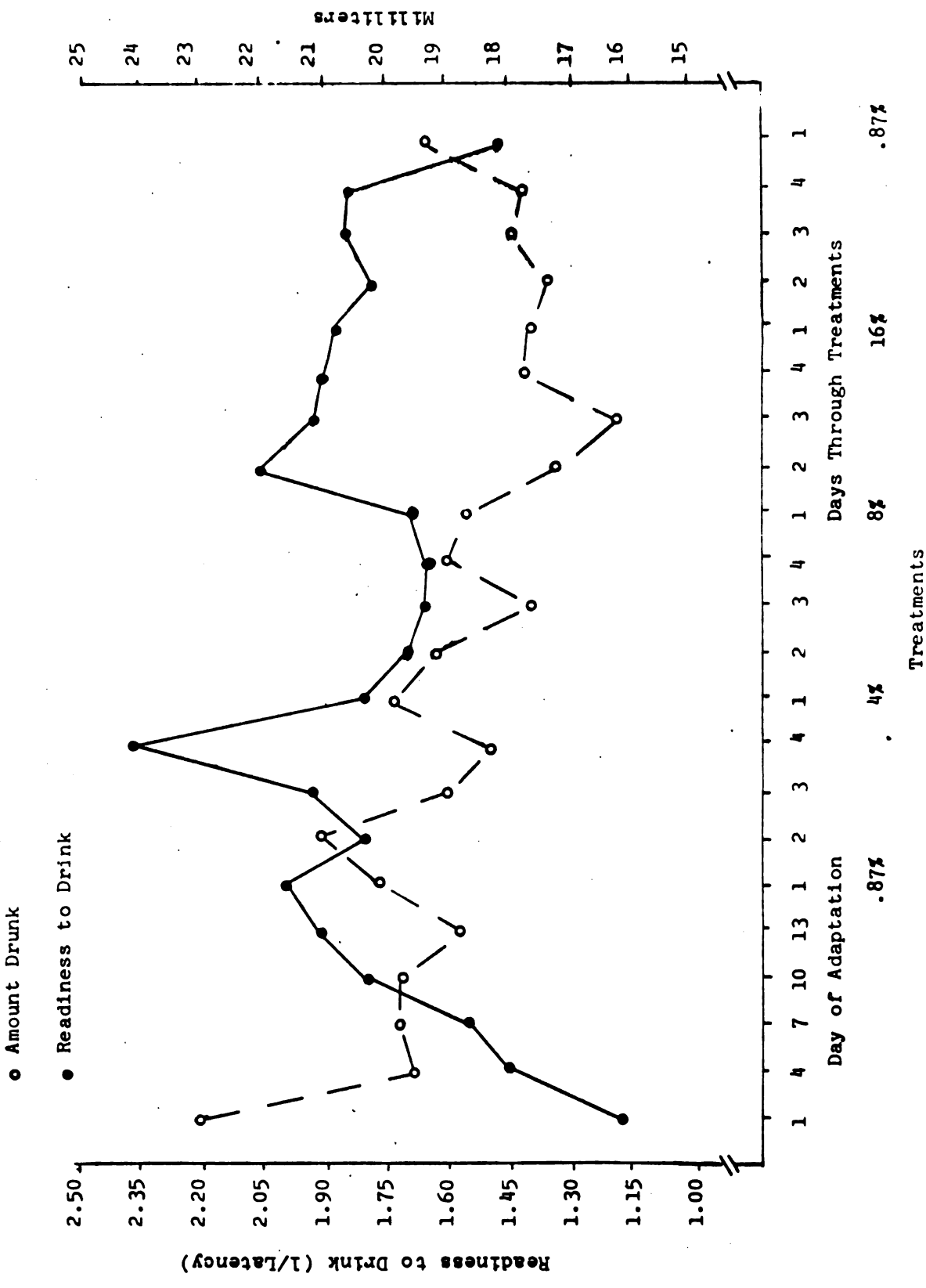

o Amount Drunk
● Readiness to Drink
Readiness to Drink (1/Latency)
Milliliters
2.50
2.35
2.20
2.05
1.90
1.75
1.60
1.45
1.30
1.15
1.00
25
24
23
22
21
20
19
18
17
16
15
1 4 7 10 13 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1
Day of Adaptation
Days Through Treatments
.87%
4%
8%
16%
.87%
Treatments

FIGURE 6

Percentage change in readiness to drink and amount drunk of 24 albino rats as a function of concentration of saline solutions (6 hour delay).

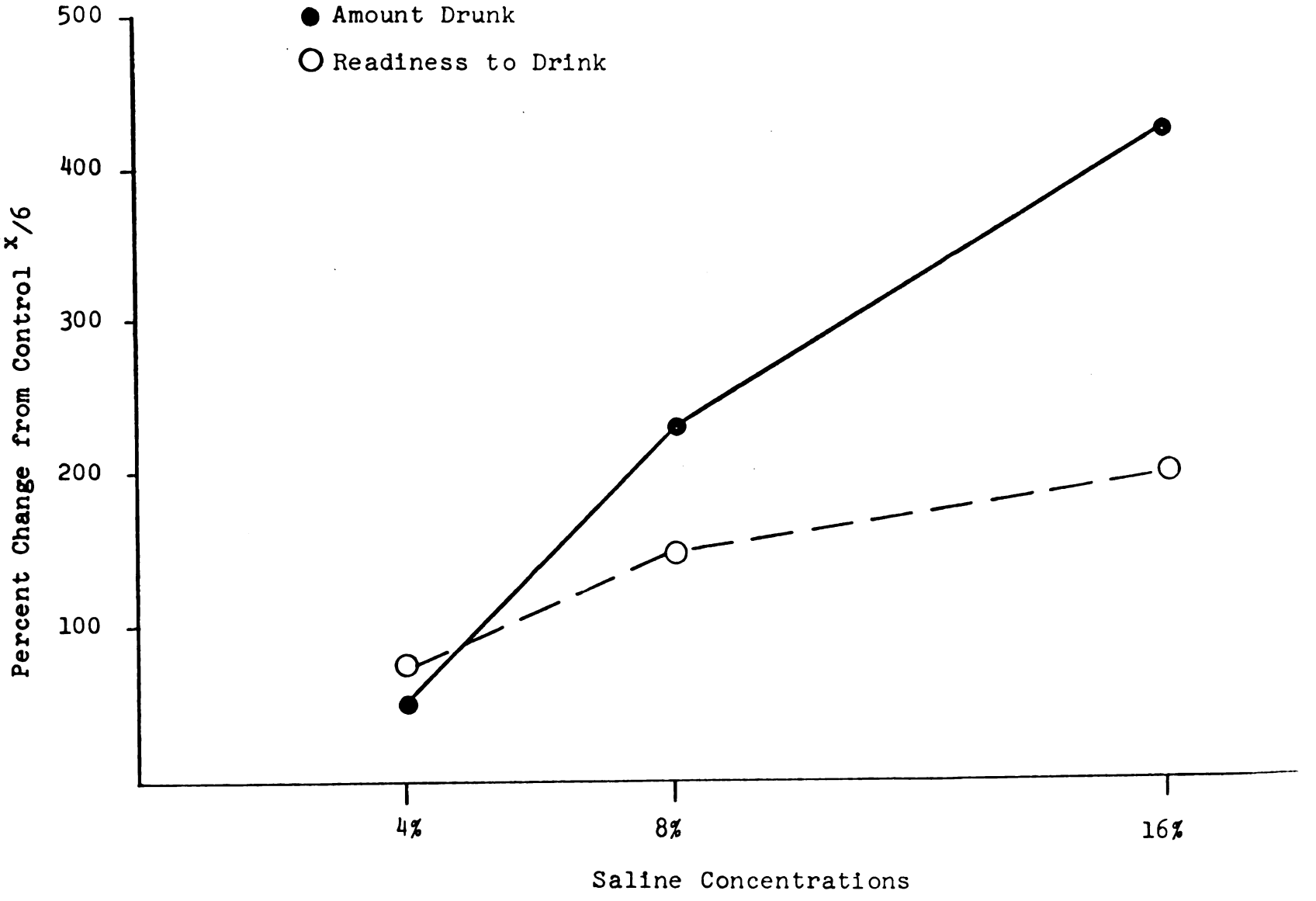

● Amount Drunk
○ Readiness to Drink
500
400
300
200
100
Percent Change from Control $\bar{x}$/6
4%
8%
16%
Saline Concentrations

TABLE 5

Summary of Analysis of Variance
(Amount drunk)

Source of Variation	DF	MS	F
Between Ss	23		
Order (O)	1	3.02	.86
Ss within gps.	22	3.52	
Withing Ss	48		
Concentrations (C)	2	11.83	29.58*
O x C	2	1.05	2.63
C x Ss within gps.	44	.40	

TABLE 6

Summary of Analysis of Variance
(Readiness to drink)

Source of Variation	DF	MS	F
Between Ss	23		
Order (O)	1	17.11	.09
Ss within groups	22	174.03	
Within Ss	48		
Concentrations (C)	2	119.79	3.71*
O x C	2	6.01	.18
C x Ss within groups	44	32.23	

Duncan's test resulted in the following information: Ss given 4% injections were less ready to drink than 16% Ss ($p < .05$). The other pairs of means did not differ significantly ($p > .05$).

The mean difference between amount drunk after injection of 4% and .87% saline was significant, ($t = 4.04$, $df = 22$, $p < .05$).

The mean difference between readiness to drink after injection of 4% and after injection of .87% saline is significant ($t = 4.07$, $df = 22$, $p < .05$).

Discussion

After delaying the availability of water for six hours following subcutaneous injections of saline solutions, the mean amount of water that rats consume increased with an increase in the concentration of the saline solution. A similar function was obtained by Wayner (1964).

Given this six hour delay, the mean readiness to drink after injection of 16% saline solution was greater than the mean readiness to drink after 4% solution. Wayner (1964) showed that when 1 meq. of NaCl was injected, the mean amount drunk for increasing delays does not change, but when 2 meq. were injected the mean amount drunk decreased up to 4 hours, then increased, indicating that changes in higher concentrations over time might result in differences after six hours between 4% and 16%. These injections were closest to those Wayner used. Actually no prior information is available relating readiness to drink and delay time following injections.

GENERAL DISCUSSION AND SUMMARY

The relationship between various concentrations of subcutaneous saline injections and readiness to drink and amount of water consumed was investigated.

In Experiment I, when access to water was given immediately following injection, amount drunk was an increasing monotonic function of increasing concentrations of saline injections. Readiness to drink was constant across saline injections.

These results were interpreted as indicating that rats could discriminate degree of dehydration produced by these means, if amount of water consumed is used as a measure. Mean readiness to drink as a measure of discrimination under these conditions did not discriminate injection conditions, whereas the decreasing variance of readiness to drink with increasing concentrations indicated differences.

In Experiment II, readiness to drink and amount drunk were measured after a six hour delay following injection and differed somewhat from the findings in Experiment I. Delay time resulted in a depression of readiness to drink for 4% NaCl injection, 8 and 16% injections remained similar in both experiments. These results are interpreted as evidence that some internal regulatory mechanism is adequate in the rat to adjust

injections of low concentrations. This has been shown to be the case with solutions of low concentrations and similar delays, when the solutions were administered intragastrically (Hatton & O'Kelly, 1966). The same relationship as in Experiment II, that is, these two injections did not differ on the readiness to drink measure, but differed in the predicted direction on the amount drunk measure, was demonstrated.

An indication of a relationship between behavioral and electrical physiological data was evidenced by the finding that when conditions of injections were comparable, the time between injections and drinking approximated the time between injections and electrical changes in certain parts of the hypothalamus. Further research is necessary to establish this as a causal relationship.

Finally, the differences between readiness to drink and amount of water consumed indicates differences between two components of drinking behavior: initiation of drinking and duration of drinking.

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APPENDICES

APPENDIX A

Apparatus

Description of the drinking boxes.

The drinking box was made up of six drinking compartments. Six glass collecting tubes were attached to the drinking box. The drinking compartments were 1 3/4 inches above a layer of sawdust. The bottom of each drinking compartment consisted of 1/2 inch hardware cloth. Each drinking compartment was 11 3/4 inches long, 5 1/2 inches wide, and 7 3/4 inches deep. The hole through which the drinking spout extended was 2 1/2 inches from the bottom of the drinking compartments, and 2 1/4 inches from the sides of the drinking compartment. The drinking spout extended one inch into the drinking compartment. The drinking spout was coupled to the gas collecting tube by rubber tubing. The tops of the drinking compartments were hinged to the compartments and were made of plexiglass. The gas collecting tubes were 27 inches long. The tubes were calibrated in .2 milliliters.

Description of the restrainer

The restrainer was a two section wood block. The top section was hinged to the bottom section. The top section was shorter than the bottom section. Thick sponges were glued to the bottom of the top section and to the top of the bottom section. The rat was placed on the sponge of the bottom section, and the top section was pulled down

around the rat and secured to the bottom section. A 2 x 2 inch hole in the top section permitted access to the rat for subcutaneous injections.

APPENDIX B

Raw Data

Experiment I

Latency to drink after saline injections.

Rat #	.87%	4%	8%	16%	.87%
			Ascending		
1	2,922	832	337	305	320
2	382	220	1,025	320	595
3	587	284	305	310	315
7	3,079	1,460	1,437	255	800
8	2,676	345	275	400	1,145
9	330	435	300	235	3,600
13	137	451	236	345	1,326
14	292	3,591	1,706	555	19,800
15	2,635	1,237	435	300	504
19	6,176	1,804	307	119	110
20	524	311	365	273	330
21	297	375	294	206	395
			Descending		
4	690	404	301	315	495
5	2,738	647	1,051	490	2,090
6	1,219	486	463	555	3,855
10	618	279	228	255	5,330
11	1,115	247	341	130	150
12	1,160	496	209	220	215
16	1,308	552	232	240	306
17	11,708	438	300	270	660
18	1,772	381	433	690	632
22	313	495	232	440	2,775
23	744	258	321	480	400
24	4,350	324	309	175	510

Experiment I

Amount drunk after saline injections.

Rat #	.87%	4%	8%	16%	.87%
			Ascending		
1	1	.4	4.8	9	1
2	1	6.2	8.6	9.4	2
3	1	3.8	6.8	10.0	2.2
7	1	2.2	2.4	7.8	.4
8	2	4.0	6.0	7.6	2.6
9	1	4.6	9.4	9.4	2.8
13	8	4.6	6.6	10.0	1.4
14	8	2.6	7.8	11.0	.6
15	10	3.0	4.6	14.6	3.2
19	1	2.0	4.6	11.0	1.4
20	1	3.4	5.8	7.0	.6
21	7	5.6	8.0	10.2	.2
			Descending		
4	1	10.4	6.6	2.4	1.0
5	1	10.0	6.0	1.8	1.6
6	5	6.8	2.0	2.2	2.4
10	2	10.4	2.0	2.2	2.4
11	4	9.8	4.6	3.6	1.8
12	2	8.0	2.8	1.8	1.2
16	5	11.4	9.2	3.2	1.6
17	2	10.8	9.6	3.0	.4
18	4	11.5	8.6	3.6	1.2
22	1	8.6	4.8	2.4	.8
23	4	11.4	5.6	3.4	2.4
24	2	11.6	8.6	4.6	2.6

Experiment I

Comparison of amount drunk before injection on treatment day and amount drunk day after injection for each saline concentration (in milliliters).

	4%		8%		16%	
Rat #	T	Day After	T	Day After	T	Day After
1	20.8	15.4	18.0	10.2	21.2	14.2
2	21.8	17.6	21.4	9.8	23.8	15.6
3	19.2	14.6	15.8	12.4	16.8	15.0
7	18.0	15.4	21.0	18.0	17.0	18.2
8	23.0	15.2	23.2	17.2	18.2	18.0
9	22.0	14.0	20.0	16.9	19.3	19.1
13	22.0	17.4	21.2	5.0	21.6	19.6
14	18.4	14.6	16.4	3.2	21.8	17.8
15	26.8	17.8	25.0	20.4	26.8	24.2
19	22.0	20.0	24.0	19.0	19.2	16.6
20	18.4	16.6	13.6	15.8	20.0	15.0
21	25.0	17.0	18.2	13.0	18.6	16.2
4	23.0	14.0	18.0	11.2	17.2	13.6
5	19.5	14.6	16.0	10.2	19.6	13.6
6	24.4	21.0	22.0	14.8	22.2	20.4
10	20.2	14.0	22.6	21.0	19.2	19.4
11	16.2	11.6	22.0	18.6	15.0	16.0
12	20.0	13.8	18.0	17.0	15.2	15.0
16	18.2	15.6	19.0	2.0	20.8	19.4
17	21.6	18.0	19.6	4.6	23.0	21.0
18	18.2	19.0	16.8	3.2	23.2	19.6
22	22.6	18.2	21.6	19.0	21.0	14.2
23	22.4	18.8	21.2	17.0	23.2	21.6
24	26.6	20.0	24.4	16.8	23.6	19.2

Experiment II (6 hour delay)

Latency to drink after saline injections

Rat #	.87%	4%	8%	16%	.87%
			Ascending		
1	31.20	.20	.65	.60	2.40
2	.60	.60	.50	.50	1.40
3	70.20	38.50	5.00	.45	6.90
7	1.00	.50	.70	.55	.85
8	1.05	.60	1.40	.60	14.85
9	.70	.40	.75	.55	11.45
13	10.40	24.85	.55	.50	29.00
15	9.90	24.70	1.50	1.40	2.80
19	2.20	.55	.50	.50	1.10
20	12.30	.70	.50	.55	4.00
21	.80	6.80	.45	.65	9.20
			Descending		
4	1.50	.70	.55	.70	27.15
5	1.10	.40	.30	.60	2.25
6	15.60	.40	.40	.50	.75
10	1.20	.35	.50	.50	.75
11	.55	.40	.45	.50	.90
12	28.35	.35	.50	.60	10.40
16	11.90	.90	.40	.65	.80
17	13.20	.55	.40	.60	.80
18	.70	.60	.50	.70	.80
22	14.00	.55	.55	.55	13.05
23	.95	.40	.50	.40	1.80
24	.95	.65	.50	.90	17.90

Experiment II

Amount drunk after saline injections.

Rat #	.87%	4%	8%	16%	.87%
			Ascending		
1	4.6	7.8	13	12.4	4.0
2	7.0	8.0	10	13.0	7.4
3	4.8	5.8	9.8	11.8	5.4
7	.8	10.4	17.4	20.6	10.8
8	5.6	10.2	14.6	15.4	8.6
9	4.4	4.6	9.6	17.0	4.2
13	5.4	7.8	14.0	12.8	5.6
15	9.4	5.6	11.0	14.6	5.8
19	12.6	10.0	12.8	9.2	8.8
20	7.2	10.6	24.8	13.8	10.0
21	6.0	11.0	16.2	19.2	10.6
			Descending		
4	7.4	12.2	5.8	5.4	5.0
5	5.6	15.2	10.8	5.8	5.8
6	5.4	12.2	7.2	6.0	6.0
10	10.6	18.8	16.0	16.8	8.8
11	5.0	15.0	13.8	9.8	6.8
12	5.6	17.2	10.0	6.2	6.4
16	6.8	15.4	13.0	8.8	8.0
17	.8	13.6	10.0	5.0	6.0
18	10.6	15.6	13.4	9.0	6.6
22	9.4	18.0	13.6	14.2	8.8
23	8.2	17.4	9.6	12.4	10.0
24	5.8	17.2	9.6	7.4	10.6

APPENDIX C

Individual Comparisons

Experiment I

Latency to drink after saline injections in quartiles (in seconds).

Rat #	4%	Rat #	8%	Rat #	16%
2	220	12	209	19	119
11	247	10	228	11	130
23	258	16	232	24	175
10	279	22	232	21	206
3	284	13	236	12	220
20	311	8	275	7	235
24	324	21	294	16	240
8	345	9	300	7	255
21	375	17	300	10	255
18	381	4	301	17	270
4	404	3	305	20	273
9	435	19	307	15	300
17	438	24	309	1	305
13	451	23	321	3	310
6	486	1	337	4	315
22	495	11	341	2	320
12	496	20	365	13	245
16	552	18	433	8	400
5	647	15	435	22	440
1	832	6	463	23	480
15	1237	2	1025	5	490
7	1460	5	1051	14	555
9	1804	7	1437	6	555
14	3591	14	1706	18	690

Experiment II (6 hour delay)

Latency to drink after saline selections in quartiles (in seconds).

Rat #	4%	Rat #	8%	Rat #	16%
1	.30	5	.30	23	.40
10	.35	6	.40	3	.45
12	.35	16	.40	2	.50
9	.40	17	.40	19	.50
5	.40	11	.45	6	.50
6	.40	21	.45	10	.50
11	.40	2	.50	11	.50
23	.40	19	.50	13	.50
7	.50	20	.50	7	.55
19	.55	4	.50	9	.55
17	.55	10	.50	20	.55
22	.55	18	.50	22	.55
2	.60	23	.50	1	.60
8	.60	12	.50	8	.60
18	.60	13	.55	5	.60
24	.65	22	.55	12	.60
20	.70	24	.55	17	.60
4	.70	1	.65	21	.65
16	.90	7	.70	16	.65
21	6.80	9	.75	4	.70
15	24.70	8	1.40	18	.70
13	24.85	15	1.50	24	.90
3	38.50	3	5.00	15	1.40