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A Mineralocorticoid-Dependent Effect of Prestress on Avoidance Responding in Rats

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A MINERALOCORTICOID-DEPENDENT EFFECT OF PRESTRESS ON AVOIDANCE RESPONDING IN RATS

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by

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PREFACE

Of the many people who helped me in this research, and in other aspects of my education, special thanks are due to Drs. Jay Weiss, Bruce McEwen, Neal Miller, and George Wolf.

Jay Weiss, my principal advisor, played an essential role in all aspects of the work described in this report. His own research and suggestions provided much of the original inspiration for this work, and his advice and encouragement were what kept it going. Moreover, if this report is readable, it is largely due to Jay's very careful readings of the earlier drafts and his many stylistic suggestions.

Brue McEwen spent many hours tutoring me in biochemistry, cell biology, and endocrinology. He also taught me basic biochemical laboratory techniques, and made his laboratory available whenever I needed it.

Neal Miller often found time from his busy schedule to advise me in this research, and in other aspects of my development as a scientist. His common sense approach to difficult scientific questions, and his insistence on not hiding behind complicated jargon and subtle hypotheses, have been a great inspiration to me.

George Wolf gave me some very important suggestions on the execution of this research. It was he who first suggested that I test sodium-loaded intact rats (Experiments 11 and 12 of this report) for the effect of prestress, a suggestion which enabled me to find that the hormonal phenomenon I was studying is not limited to the adrenalectomized rat.

ABSTRACT

Rats made more free-operant avoidance responses if they were given a brief prestress 30 min before the test than if they were undisturbed before the test. This effect of prestress occurred in normal rats whether foot shock, air blast, or simple handling served as the prestress. The effect did not, however, occur in adrenalectomized rats. The effect also did not occur in intact rats who were maintained on 1.5% NaCl drinking fluid, a procedure which is known to inhibit mineralocorticoid secretion. Injection of mineralocorticoid, in either adrenalectomized rats or intact NaCl-maintained rats, renewed the effect of prestress and had no effect on the subjects' response rates in sessions not preceded by prestress. Prestress completed only 1 min, rather than 30 min, before the session had a different type of effect on avoidance responding, and this effect was not abolished by adrenalectomy. It is concluded that the presence of mineralocorticoid is required for the development of a particular delayed behavioral reaction to environmental disturbance.

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INTRODUCTION

There have been many recent studies of the influence of pituitary and adrenal hormones on avoidance behavior in rats. These studies have relied largely on two general types of experimental procedures. One procedure has been to inject intact rats with a particular hormone, and to compare the injected rats, in avoidance performance, with sham-injected controls. The other has been to surgically (e.g. by adrenalectomy or hypophysectomy) deplete rats of a particular group of hormones, and to compare the depleted rats, in avoidance, with either intact controls or other operated rats given hormone replacement. As shown in a recent review by di Giusto, Cairncross, and King (1971), these procedures have resulted in relatively consistent effects of ACTH, glucocorticoids, and perhaps also epinephrine, on avoidance performance.

A fundamental difficulty in interpreting the results of these studies, however, lies in determining whether the observed effects are normal, physiological, effects. The rationale for using hormones of the pituitary-adrenal axis, and of the adrenal medulla, is that these are secreted in response to stress, and therefore might play a role in modifying a normal rat's behavior following stress. But injected hormone may not truly mimic the physiological stress release of the hormone; and glandectomy undoubtedly does more than merely prevent release of the hormone in question.

I therefore chose a different approach to the problem of hormonal involvement in avoidance. Instead of using a hormone as the primary independent variable, I used stress as the primary variable. I first asked the question: Does a normal rat's behavior in an avoidance task depend, in any way, upon whether or not he was stressed shortly before beginning the task? When the answer to this question was affirmative, I then asked a second question: Does this normal effect of stress depend upon a particular hormone? This approach differs from the usual procedures, above, in that surgery and hormone

injections were not used to establish an effect on avoidance performance, but were only used to investigate the mechanism of an effect which was first shown to occur, as a result of stress, in intact normal rats.

The results were surprising. They did not support the hypothesis that stress-induced hormonal release plays a role in avoidance. Instead they established a different, unexpected, type of hormonal dependence for an effect of acute stress on avoidance behavior.

Rats made more avoidance responses in a session if they were subjected to any of a wide variety of brief stressors, 30 min before the avoidance session, than if they went directly into the avoidance apparatus from an undisturbed state. This effect of prestress, while very reliable in normal rats, completely failed to occur in adrenalectomized rats. With further experiments, the effect was found not to depend upon the adrenal's stress hormones (glucocorticoids or catecholamines), but rather to depend upon mineralocorticoids, the salt-regulating hormones of the adrenal! The effect of prestress on avoidance could be abolished by maintaining intact rats on high sodium diet, a procedure which inhibits mineralocorticoid production, as well as by adrenalectomy. And in either sodium-loaded or adrenalectomized rats, injection of a mineralocorticoid renewed the prestress effect. While mineralocorticoid permitted the effect of prestress to occur, it had no effect on the subjects' baseline avoidance response rates, when no prestress was given. The present report describes the experiments which led to these conclusions.

To measure avoidance behavior in all of these experiments, a free-operant avoidance task was used, in which the subject was continuously free to respond (by turning a wheel), and each response postponed onset of electric shock. An important advantage of the free-operant task is that it provides no physical restraints on when, or when not, the subject may respond in a session, and thereby permits experimental effects to appear at any time. The effect of prestress on avoidance responding was expected to change as a function of time

in the avoidance session, and the free-operant paradigm permitted the detection of this change.

For the purpose of this report, the experiments are grouped into three separate categories. The first includes experiments which established and described the effect of prestress on avoidance in normal rats. The second includes experiments which established that the effect does not depend upon the stress-induced release of pituitary or adrenal hormones, but does depend upon the presence of mineralocorticoid. And the third includes experiments which investigated the influence of time between prestress and the avoidance session on the behavioral effect, and on the mineralocorticoid-dependence of the effect. These categories of experiments are presented after an initial Methods section.

METHODS

These methods apply, generally, to the various experiments in this report. When additions and exceptions to them occur, they are given later, in the texts for each individual experiment.

Subjects and Surgery

All subjects were adult, male, Sprague-Dawley rats, purchased from the Charles River Breeding Company. In all, 145 'normal' 56 adrenalectomized (adrex), and 10 hypophysectomized (hypox) rats served as subjects. The ranges in body weights, taken for each subject on the last day of service in an experiment, were: 347-570 g for normal, 332-484 g for adrex, and 221-318 g for hypox subjects. All potential subjects received several training sessions in the avoidance task before starting an experiment, and those who failed to learn the task, or who were particularly inconsistent in their response rate, were excluded before beginning an experiment. In all experiments combined, fewer than 20% of either normal or adrex potential subjects, but 55% of hypox potential subjects, were excluded for this reason. With only one exception (Expt. 3), the subjects for no experiment had served in a previous experiment.

The adrenalectomies were performed by a dorsal approach under pentobarbital or ether anesthesia. To avoid leaving any cells which could initiate regeneration of adrenocortical tissue, the adrenals were removed with capsules intact and with much of the surrounding fat still attached. Sham adrenalectomies were performed on the 'normal' subjects for two experiments (Expts. 14 and 15). These were done in the same way as were adrenalectomies except, of course, that the adrenals were not removed. All adrenalectomies and sham adrenalectomies were performed at least 20 days before the beginning of an experiment, and at least 10 days before the first day of training. The hypophysectomies were done, by a pharyngeal approach, by the breeding company (Charles River) at least 3 months before the hypox rats were trained for an experiment.

Housing and Diet

Each subject, in every experiment, was housed individually in a stainless steel cage, 12 x 12 x 7 inches, which had solid walls and floor, and a wire mesh cover. Wood shavings served as litter on the floor of each cage. To reduce possibly stressful effects of auditory stimuli reaching the animal room, each home cage was kept inside an outer cardboard box lined with Fiberglas insulation. The outer boxes varied in construction, but all of them were about 1 1/2 ft in each dimension, had removable tops, and had numerous small holes in the tops and sides for ventilation.

The animal room, in which all subjects were housed, was used for no other purpose. A constant attempt was made to minimize noise in it, and the temperature was maintained within the range 72 to 76° F. For Experiments 1, 2, and 3, the room lights were kept on from 5:00 a.m. to 5:00 p.m. and off from 5:00 p.m. to 5:00 a.m.; for all other experiments, they were kept constantly on and unvaried in intensity. Because of the insulated boxes, the illumination which reached the subjects when the room lights were on was relatively dim.

All subjects had Purina Lab Chow as their only food, and this was continuously available in their home cages. Except for the experiments involving saline maintenance of normal subjects (Expts. 11 and 12), all normal and hypox subjects had tap water as their only source of fluid. All adrex subjects had a saline solution for drinking, from the day of the operation to the end of the experiment. The adrex subjects of one experiment (Expt. 7) had only a single fluid, 1% NaCl solution, to drink; but those of all other experiments were maintained on a continuous choice, between tap water and 3% NaCl.

Avoidance Task

The avoidance chamber was a transparent Plexiglas box, 9 1/2 x 8 1/2 x 17 inches, with a grid floor, through which electric shock could be delivered, and a wheel mounted on one wall which the rat could rotate to turn off or postpone electric shock. The grids were 1/8 inch

dia. stainless steel dowels positioned 1/2 inch center to center. The wheel consisted of two parallel steel disks, 4 inches dia., positioned 2 inches apart with an axle through the center and 24 steel dowels, each 1/8 inch dia., equally spaced around the periphery. It was mounted on the outside of the box, but protruded 3/8 inch into the box, at which point it was 3 1/4 inches above the grid floor. The rat could turn the wheel on its axle with very little effort by pushing down, almost invariably with one or both forepaws, on one of the peripheral cross-dowels of the wheel. Every 1/8 revolution of the wheel momentarily closed a microswitch which caused the recording of one response and the offset or delay of electric shock. The shock was provided by a high voltage, constant current, AC shock source (Electrocraft of Canada, NE 101 Shock Generator), and was delivered through neon bulbs (General Electric, NE2) wired in series across the grids of the floor. All shocks were delivered, and data recorded, automatically by means of electromechanical programming equipment.

Three avoidance chambers were available, and each was kept inside a Lehigh Valley sound-dampening outer box provided with an electric blower for ventilation and a 7 1/2 Watt lamp inside for illumination. The "apparatus room," in which the avoidance tests took place, was separate from, but adjacent to, the animal room.

During an avoidance session, shock was initiated whenever, and only when, the subject allowed 5 sec to elapse without making a response. The shock was presented in pulses consisting of 0.5-sec shock-on periods separated by 0.05-sec (approximately) shock-off periods. The shock was terminated only when a response occurred. A response did not cut short an on-going shock pulse, so the minimal duration of each shock was 0.5 sec. The current level of each shock was 0.5 ma.

Only those responses which occurred more than 2 sec after offset of a shock were recorded as avoidance responses (ARs). Any response which turned off shock or which fell within 2 sec of shock offset was considered an escape response and recorded separately. Other data

recorded were the number of shocks initiated and the total duration of shock-on periods. All data were accumulated and recorded separately for each 15-min portion of each avoidance session. ARs generally yielded the most reliable experimental effects, and are the principal data used in this report.

Prestress

The basic independent variable in these experiments was prestress (PS) vs no prestress (NPS). The usual PS was two brief electric foot shocks given 30 min prior to beginning the avoidance session. The procedure for this was as follows: the subject's home cage was lifted out of the insulated box and carried into the apparatus room; the rat was lifted from his home cage into a "prestress chamber" where he received 2 inescapable electric shocks, each of 0.5 ma current and 1 sec duration, one coming after 30 sec in the chamber and the other after 90 sec; and then, after a total of 2 min in the prestress chamber, the rat was returned to his home cage and then to his insulated box in the animal room. The procedure required a total of about 2 1/2 min to complete from the time the subject's insulated box was opened to the time he was put back in it. The prestress chamber was similar to the avoidance chamber except that it had no wheel, its walls were black rather than transparent, and it was not kept inside an outer box. It had the same type of grid floor and shock supply as the avoidance chamber.

Other prestress procedures were also used, and are described in the texts for individual experiments. The procedure for NPS was simply to leave the subject undisturbed in his home cage until the avoidance session was begun. In either the NPS or the PS condition, the avoidance session was begun by carrying the subject's home cage, with subject inside, into the apparatus room, placing the subject into the avoidance chamber, and starting the avoidance apparatus. This required 15-20 sec, from the opening of the subject's insulated box to the start of the avoidance apparatus.

Experimental Design

All subjects were trained in the avoidance task, and received at least 5 hours' experience in the task, extending over at least 7 different daily sessions, prior to starting an experiment. In most experiments, subjects were tested for one session per day throughout the experiment, and were alternately given PS before one day's session and NPS before the next. This design permitted comparison of each subject's response rate in PS sessions with his own response rate in NPS sessions, thus allowing each subject to serve as his own control. The order of presentation of PS and NPS was counterbalanced across subjects in each experiment; half of the subjects began the alternation with PS and the other half began with NPS. A given subject was always run in the same apparatus, and at the same time of day, every time he was tested.

Summary of Abbreviations

AR - Avoidance Response

PS - Prestress

NPS - No Prestress

EFFECT OF PRESTRESS DELIVERED 30 MIN BEFORE AVOIDANCE SESSIONS IN NORMAL RATS

Experiment 1

This experiment was the first test for the effect of prestress on avoidance behavior, and resulted in the first observation of the phenomenon on which the succeeding experiments were based. Seven subjects were used, and each was tested in the avoidance task for one session per day, for 10 days, alternately being given prestress before one day's session and not before the next. Three subjects (N2, N4, and N7) began the alternation with PS and ended with NPS, and four began with NPS and ended with PS. The PS was the foot shock prestress described in Methods, and it was completed 30 min before the avoidance session began. The length of each avoidance session was 30 min, and the data were accumulated separately for the first and second 15 min portions of each session. The sessions took place between 7:00 a.m. and 11:00 a.m.

Results

The mean number of ARs each subject made in each 15 min portion of NPS and PS sessions is shown in the lefthand columns of Table I. It can be seen that there was great variability among subjects in the actual number of ARs made, but there was consistency in two effects. One of these is a within-session warmup effect, which can be seen in the table by the fact that every subject made more ARs in the second 15 min portion of the session than in the first 15 min portion, regardless of whether or not prestress was employed. Warmup is a common finding in free-operant avoidance (e.g. Wertheim, 1965), and this effect was not the main focus of the present study. The other effect, which was the main focus, is a prestress effect. The prestress effect, as seen in Table I, appeared in the second 15 min portion of the session, not the first, and is indicated by the fact that all but one subject made more ARs in the second 15 min portion of PS sessions than in the second 15

Table I

Avoidance Response Data for Individual Subjects in Expt. 1

	Mean ARs				Total	% of Total			
Subject	1st NPS	15 min PS	2nd NPS	15 min PS	of Means	1st NPS	15 min PS	2nd NPS	15 min PS
N1	59	45	92	166	362	16.3	12.4	25.4	45.9
N2	78	74	214	263	629	12.4	11.8	34.0	41.8
N3	67	162	247	462	938	7.1	17.3	26.3	49.3
N4	797	1047	1354	1522	4720	16.9	22.2	28.7	32.2
N5	770	744	1660	1920	5094	15.1	14.6	32.6	37.7
N6	69	52	197	185	503	13.7	10.3	39.2	36.8
N7	37	79	190	267	573	6.5	13.8	33.2	46.6
X						12.6	14.6	31.3	41.5
SE						1.6	1.5	1.8	2.3

min portion of NPS sessions. There is no indication of a prestress effect in the first 15 min portion of the session; three subjects made more ARs here in PS than in NPS sessions, and four made the reverse.

The prestress effect is more easily visualized, and more conveniently dealt with statistically, if the AR data are transformed into percentage scores and averaged over the seven subjects. This transformation is shown in the righthand columns of Table I. For each subject, the number of ARs made in each 15 min portion of each type (NPS and PS) of session is expressed as a percentage of the total ARs made by that subject over both portions of both types of sessions. The means and standard errors of these percentage scores, over the seven subjects, are shown at the bottom of Table I and again, graphically, in Figure 1. The significance level (.05) shown in Figure 1, for the difference between second 15 min PS and NPS scores, was determined by a one-tailed dependent t test on the percentage data in the two righthand-most columns of Table I ($t=2.91$, $df=6$, $p<.05$).

Both Table I and Figure 1 show only the average effect of prestress for the five pairs of sessions in the experiment. It is also of interest to know whether the effect of prestress varied from one pair of sessions to the next. Therefore, another calculation was performed in which the number of ARs made by each subject in each session was expressed as a percentage of the total ARs made by the subject over all 10 sessions. Only ARs made in the second 15 min portion of each session were included for this calculation, since no effect of prestress appeared in the first 15 min portion. The mean and standard error, over subjects, of these percentage scores, for each successive NPS and PS session, is illustrated in Figure 2. It can be seen that prestress had a significant effect ($p<.05$) the first time it was used, and there is no sign that the effect either increased or decreased consistently with continued use.

None of the data other than ARs, collected in this experiment, revealed a significant effect of prestress. The mean number of shocks taken per 15 min portion of session was 105.1 for first 15 min, NPS; 105.8 for first 15 min, PS; 81.8 for second 15 min, NPS; and 74.3 for

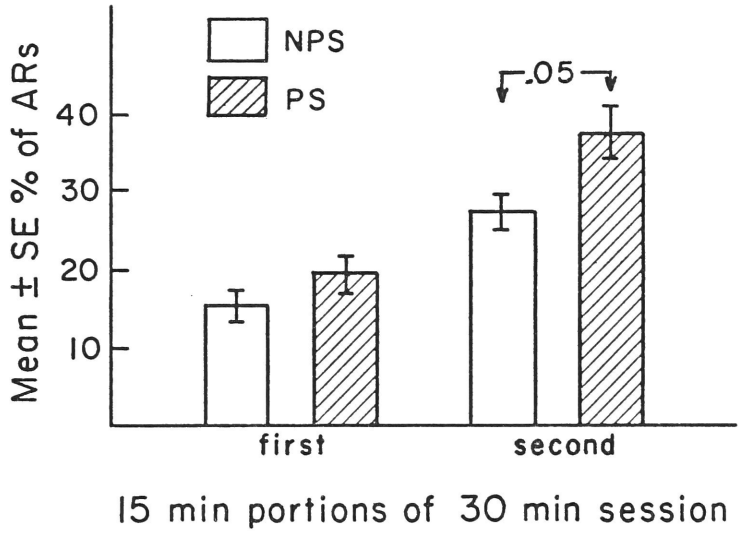

Figure 1. Mean % of avoidance responses (ARs) which occurred in each 15 min portion of non-prestressed (NPS) and prestressed (PS) sessions in Expt. 1. Subjects were normal rats, and PS was foot shock delivered 30 min before the session. The significance level (.05), given for the effect of PS in the second 15 min, was determined by a one-tailed dependent t test. This test was used for all significance levels presented in the figures of this paper.

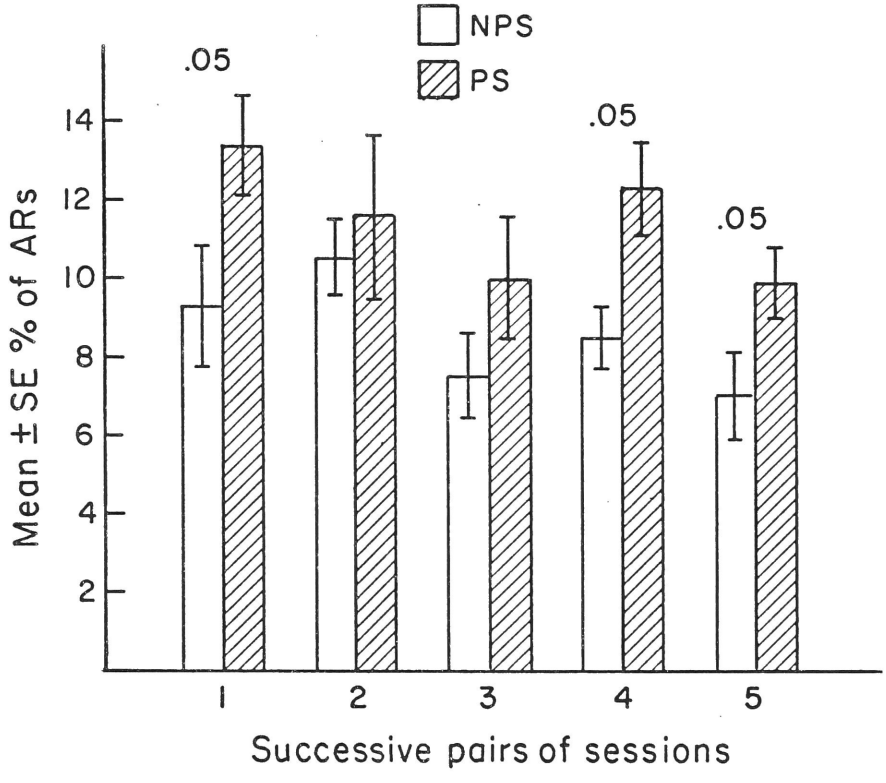

Figure 2. Mean % of ARs which occurred in the second 15 min portion of each successive NPS and PS session in Expt. 1.

second 15 min, PS. The mean duration of each shock (latency to escape) was 0.97 sec for first 15 min, NPS; 0.76 sec for first 15 min, PS; 0.69 sec for second 15 min, NPS; and 0.68 sec for second 15 min, PS. And the mean number of escape responses was 442 for first 15 min, NPS; 446 for first 15 min, PS; 358 for second 15 min, NPS; and 381 for second 15 min, PS. Each of these different measures was analyzed in the same manner as were the AR data, above, and none revealed a significant effect of prestress. The difference between second 15 min, PS, and second 15 min, NPS sessions in shocks taken, however, approached significance (dependent $t=1.74$, $df=6$, $.05<p<.10$).

It is remarkable that the effect of prestress on ARs occurred in the second, and not the first, 15 min portion of the avoidance session. During the first 15 min portion of the session, each subject received approximately 100 shocks, each as strong as the 2 shocks used for the prestress; yet only after this did the effect of the prestress appear. This delayed appearance of the prestress effect occurred repeatedly in succeeding experiments, and is a reliable result.

Experiment 2

Is the prestress effect, observed in Experiment 1, a result of the general stressor properties of the prestress, or is it a result of some specific information imparted to the animal by the prestress? If the effect is mediated by the release of ACTH and glucocorticoids, or by any other aspect of the general physiological stress response, then any acute stressor, not just foot shock, used as the prestress, should result in the prestress effect. On the other hand, if the effect depends upon some more specific information imparted by the prestress, then only certain stressors, those containing the essential information, or cue content, should be capable of causing the prestress effect. The purpose of Experiment 2 was to determine whether a prestress procedure very different from the foot shock prestress used in Experiment 1 would nevertheless have the same effect on avoidance responding.

A notable characteristic of the foot shock prestress used in Experiment 1 is that it greatly resembled, in cue content, the avoidance session itself. Both prestress and initiation of the avoidance session involved removal of the rat from his home cage, placement of him into a Plexiglas chamber with a grid floor, and delivery of electric foot shock. The possibility arose that such similarity is essential for the prestress effect to occur. Therefore, for Experiment 2, a prestress procedure was used which was very different from the avoidance procedure as well as from the foot shock prestress procedure.

This prestress was a noisy blast of air, delivered from the outflow side of a Kenmore canister-type home vacuum cleaner, into the subject's home cage. In order that the prestress for a given subject would not disturb the other subjects, the subject's entire insulated outer box, with home cage inside, was first carried out of the animal room into the apparatus room. Then the cover of the insulated box was partially opened and the vacuum cleaner hose was pushed into the box so that the nozzle rested on the wire cover of the cage and pointed down into the cage. The vacuum cleaner was then turned on for two 5-sec

periods separated by 1 min, and the hose was then removed from the box and the box, with home cage and subject still inside, was returned to the animal room. This entire prestress procedure required about 2 1/2 min to complete. The delay between completion of the prestress and initiation of the avoidance session was 30 min, the same as in Experiment 1.

Tests of this prestress on several non-experimental rats, with outer-box covers completely opan for observation, indicated that the air blast, and/or its accompanying loud sound, caused the rat to show definite emotional responses. Typically the rat would first run rapidly in circles around the cage and then would crouch motionless and defecate.

Ten rats served as subjects; they were tested every day for six successive days, alternately receiving the air blast PS before one day's session and NPS before the next. Five subjects started the alternation with PS and five started with NPS. The session length was 30 min. All sessions took place between 11:00 a.m. and 2:00 p.m.

Results

The data are illustrated in Figure 3, which is composed in the same way as was Figure 1 for Experiment 1. The air blast had the same type of effect as did the foot shock prestress, again appearing significantly in the second, but not the first, 15 min portion of the session. Analysis of the data, for individual pairs of sessions, like that employed for Experiment 1 (Fig. 2), revealed that the air blast PS significantly ($p<.05$) enhanced second-15-min ARs the first time it was used.

In summary, a prestress procedure which was designed to contain minimal stimulus similarity to the avoidance procedure, nevertheless, resulted in the prestress effect. The prestress effect appears not to depend upon specific informational content of the prestress, since two such different stressors as air blast and foot shock had the same effect.

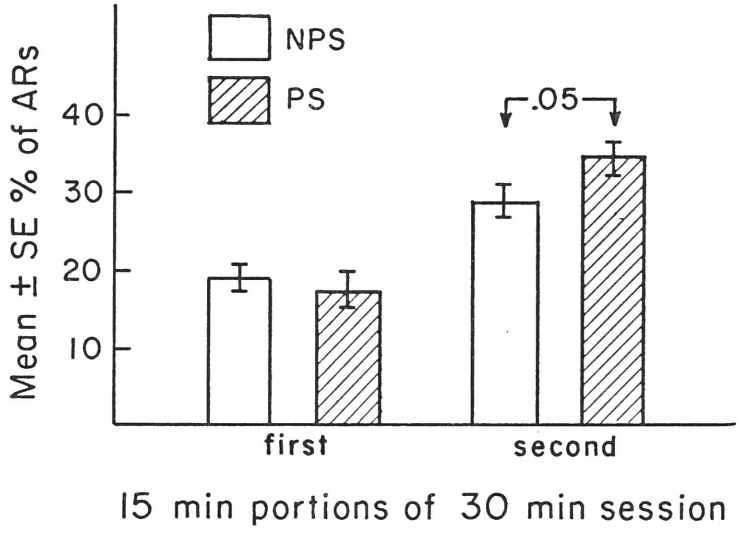

Figure 3. Mean % of ARs which occurred in each 15 min portion of NPS and PS sessions in Expt. 2. Subjects were normal rats, and PS was air blast delivered 30 min before the avoidance session into the subject's home cage.

Experiment 3

Is the prestress effect, observed in Experiments 1 and 2, simply a wake up phenomenon? In Experiments 1 and 2, the subjects were tested during the day (the lights-on portion of the animal room light cycle), when rats, being nocturnal animals, are normally inactive or sleeping. The possibility arose that prestress augmented avoidance responding simply by waking the subjects prior to the avoidance session. To test this possibility, rats in Experiment 3 were tested for the prestress effect at night, when they were most likely to be already awake.

The light cycle used in Experiments 1 and 2 (on at 5:00 a.m. and off at 5:00 p.m.) was continued in this experiment, and the subjects were tested between 6:00 p.m. and 9:00 p.m. No data were taken on home cage activity for these subjects, but other experimenters (Hunt and Schlosberg, 1939) have found that albino rats, caged individually on a 12-hr lights-on, 12-hr lights-off cycle, are much more active at this time after light offset than at any lights-on part of the day.

The subjects were the same 10 rats who had served in Experiment 2. Thirty-one hrs after completing the final session of Experiment 2, each subject was given one 30 min training session in the avoidance task at the new time of day, and the experiment was begun 24 hrs after that. Each subject was tested every day for six successive days, alternately receiving the standard foot shock PS (as described in Methods and as used in Experiment 1) before one day's session and NPS before the next. Five subjects started the alternation with NPS and five started with PS. The session length was 30 min.

Results

The results are illustrated in Figure 4. Once again, the prestress effect appeared significantly in the second 15 min portion of the session but not the first. The effect for this experiment was not smaller (in fact it was larger, though not significantly so) than the effects observed in Experiments 1 and 2. The data, therefore, lend no

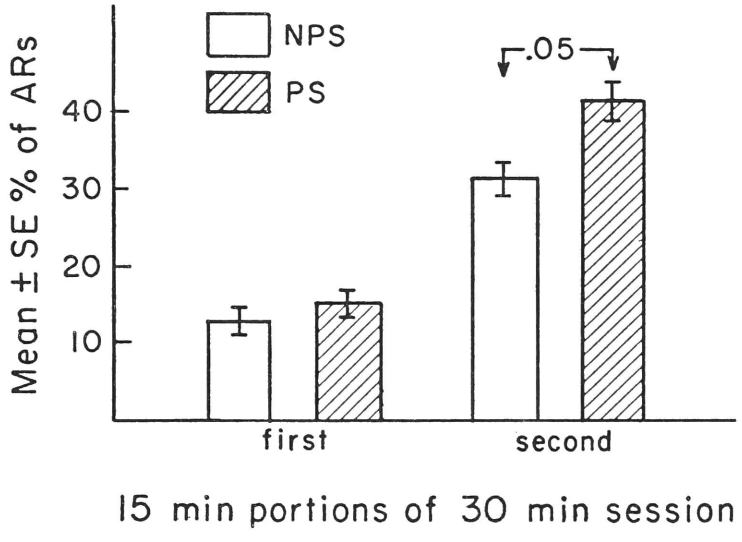

Figure 4. Mean % of ARs which occurred in each 15 min portion of NPS and PS sessions, when all sessions were conducted at night, in Expt. 3. Subjects were normal rats, and PS was foot shock delivered 30 min before the session.

support to the hypothesis that prestress has its effect by waking up a sleeping subject.

Taking Experiments 1, 2, and 3 together, the prestress effect has been shown not to depend upon the time of day, with respect to the light cycle, that the subjects were tested. For all of the remaining experiments, the lights in the animal room were left continuously on and unvaried in intensity.

Experiment 4

In Experiments 1, 2, and 3, the prestress effect occurred significantly in the second, but not the first, 15 min portion of a 30 min avoidance session. What would happen if the session were longer than 30 min? Would the prestress effect continue to get larger, would it remain about the same, or would it get smaller with further time in the avoidance apparatus? Experiment 4 was designed to test these possibilities by using 90 min avoidance sessions.

Ten new subjects were used, and each was tested on 16 consecutive days, alternately receiving PS before one day's session and NPS before the next. As always, the order of the alternation was counterbalanced across subjects. The PS was the usual foot shock PS completed 30 min before initiation of the avoidance session. The data were accumulated separately for each 15 min portion of the 90 min session.

Results

The results are illustrated in Figure 5, which was compiled in the same way as were the figures for the previous experiments except that the percentages were calculated for six, rather than two, 15 min portions of NPS and PS sessions. A significant PS-induced enhancement appeared in every 15 min portion of the 90 min session except, as usual, the first. Furthermore, there is no evidence that the effect got either larger or smaller with time between the second and sixth 15 min portions.

The prestress effect is not a transitory one; it appears even in the last 15 min portion of a 90 min avoidance session.

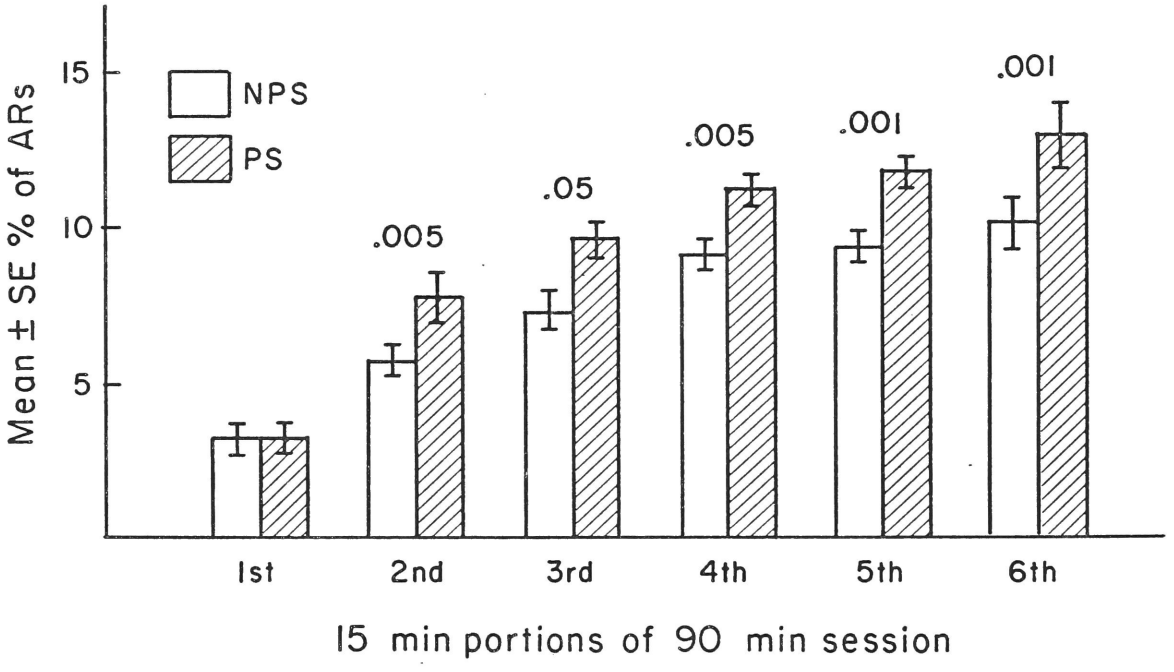

Figure 5. Mean % of ARs which occurred in each 15 min portion of NPS and PS sessions, when all sessions were 90 min long, in Expt. 4. Subjects were normal rats, and PS was foot shock delivered 30 min before the session.

Experiment 5

One of the classic ways of explaining differences in avoidance behavior involves the concept of "fear." Fear, a central motivational state, is thought to increase avoidance responding. Since the prestress effect, as observed in Experiments 1-4, is an increase in avoidance responding, the possibility arose that the effect might represent an increase in the subject's level of fear. If this is the case, and if fear is to be a useful concept in describing the prestress effect, then one should be able to use the fear concept to make certain predictions about the prestress effect.

Fear is presumably a result of the shock used in the prestress apparatus and in the avoidance apparatus. If prestress augments the rat's avoidance response rate by augmenting fear, then use of strong shock for the prestress, eliciting greater fear, should cause a larger augmentation in ARs than a mild prestress. Conversely, use of strong shock in the avoidance apparatus should raise the subject's fear level, and consequently his AR rate, independently of prestress, and should thereby diminish the possibility for additional augmentation by the prestress. In other words, the "fear hypothesis," assuming fear to be a variable affected both by prestress shock and avoidance shock, predicts that the prestress effect should vary directly with the strength of prestress shock relative to that of avoidance shock. Experiment 5 was designed to test this prediction by testing different groups of subjects at different strengths of prestress and avoidance shock.

Three levels of prestress shock and three levels of avoidance shock were used in a 3 x 3 factorial design. The three shock levels, both for prestress and for the avoidance stimulus, were 0.15, 0.5, and 1.7 ma. In a preliminary experiment, these shock levels were found to be nearly the broadest range of currents which could safely be used in the avoidance task. The 0.15 ma shock was close to the mildest which would still elicit consistent escape and avoidance responding in most rats, and the 1.7 ma shock was apparently very painful

and often elicited considerable jumping and squealing as well as escape and avoidance responding.

There were nine different groups of subjects, and each group was tested at a different one of the nine combinations of the three prestress and three avoidance shock levels. Fifty-four subjects were used in all, and they were distributed among the nine groups by a procedure which matched the different groups as nearly as possible on the basis of the subjects' AR rates in training, before the experiments was begun.[1] Except for the different shock currents used, the prestress and avoidance procedures were the ones described in Methods. Each subject was tested for one 30 min session per day, for 15 days, alternating between NPS and PS sessions.

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[1]The training and matching procedures for Experiment 5 are described in this note. After initial shaping in the avoidance task, each subject received ten 30 min training sessions, held on consecutive days, before starting the experiment. The avoidance shock current for the first three of these sessions was 0.15 ma for all subjects. The subjects were then ranked, on the basis of the number of ARs they made in the second plus third training session, and each successive group of three subjects in the ranking formed a "triplet." Each subject in a triplet was assigned, by a random procedure, to a different one of the three avoidance-shock-level groups. One group remained at 0.15 ma avoidance shock throughout the remaining training sessions, and throughout the experiment; another group was run at 0.5 ma avoidance shock throughout the remaining training sessions, and throughout the experiment; and the third group was run at 0.5 ma avoidance shock for the fourth and fifth training sessions, and then at 1.7 ma for the remaining training sessions and throughout the experiment. After the final day of training, the subjects within each avoidance-shock-level group of subjects were ranked, for a second time, on the basis of the number of ARs they made in the final two training sessions. New triplets were formed from this ranking, and each subject in a triplet was assigned, by a random procedure, to a different one of the prestress shock levels: 0.15 ma, 0.5 ma, or 1.7 ma. Three prestress-shock-level groups were thus formed within each of the three avoidance-shock-level groups, constituting a total of nine "matched groups" of subjects.

Results

To facilitate comparison of the prestress effect among the nine groups of subjects, a single percentage measure of the effect was used for each subject. This measure was basically the same as the percentage measures which were used in all of the previous experiments to express the prestress effect, except that the data from the first 15 min portion of each session were completely excluded. The number of ARs each subject made in the second 15 min portions of PS sessions was expressed as a percentage of the ARs he made in the second 15 min portions of PS plus NPS sessions. Since there were an equal number of PS and NPS sessions, a score greater than 50, by this measure, indicates a positive effect of prestress. Table II gives the mean ±SE of these percentage scores for each of the nine different groups of subjects. The significance level (*p*) given in the table for each mean was calculated by a one-tailed *t* test (*df*=5) for the difference between the observed percentage score and the chance value, 50. The mean ±SE of the percentage scores for all subjects run at a given avoidance shock level is presented at the bottom of each column, and the same information for all subjects run at a given prestress level is presented at the end of each row. The *t*, and associated significance level, given at the bottom of each column and end of each row, is the result of a *t* test (*df*=17) to the reliability that the mean for that row or column is greater than 50.

It is clear from Table II that a highly significant effect of prestress occurred for every prestress shock level (row) and every avoidance shock level (column) used in this experiment. Moreover, there is no indication that the strength of the prestress shock had any effect on the size or reliability of the prestress effect. The table does suggest that increasing the avoidance shock level tended to decrease the size of the prestress effect. But it is equally clear from the table that the standard error of the effect also decreased as the avoidance shock increased, and the reliability of the prestress effect didn't decrease. A two-way analysis of variance for matched groups, conducted on these data, failed to reveal a significant effect of

Table II

The Prestress Effect at Different Levels of Prestress and Avoidance Shock, Expt. 5

		AVOIDANCE SHOCK				
		0.15 ma	0.50 ma	1.70 ma		
PRESTRESS SHOCK	0.15 ma	56.0 ±3.2 p<.07	55.2 ±2.8 p<.07	51.5 ±2.0 p<.25	54.2 ±1.5	t=2.81 p .01
	0.50 ma	58.1 ±3.4 p<.05	56.4 ±4.2 p<.10	53.5 ±1.1 p<.02	56.0 ±1.8	t=3.43 p .005
	1.70 ma	58.6 ±6.7 p<.15	55.0 ±2.9 p<.10	55.1 ±1.7 p<.02	56.2 ±2.4	t=2.60 p .01
	$\bar{X}$	57.6 ±2.6	55.5 ±1.8	53.4 ±1.0	55.8 ±1.1	
		t=2.80 p<.01	t=3.11 p<.005	t=3.55 p<.005		5=4.96 p<<.001

avoidance shock level (F=2.47, df=2,10, $p>.10$), of prestress shock level (F=0.48, df=2,10, n.s.), or of the interaction between the two (F=0.08, df=4,20, n.s.) on the size of the prestress effect. In other words, no particular avoidance shock level, no particular prestress shock level, and no particular combination of avoidance and prestress shock levels was reliably better than any other in permitting the prestress effect to occur.

Figure 6 shows, graphically, the effect of prestress for each of the three avoidance-shock-level groups of subjects. This figure was compiled in the same way as were the figures for previous experiments, except that the percentages were taken for each 7 1/2 min, rather than 15 min, portion of the avoidance session. The reason for using 7 1/2 min portions was simply to see better how the prestress effect interacts with time in the avoidance session. It can be seen from the figure that the prestress effect was significant for both the third and fourth 7 1/2 min portions for all three avoidance-shock groups, and was generally not significant for the first or second 7 1/2 min portions. The one exception was a significant effect in the second 7 1/2 min for the 1.7 ma group. The similarity among the three avoidance-shock-level groups, shown in Figure 6, indicates that the warmup effect, as well as the prestress effect, does not depend upon the use of a particular strength of shock in the avoidance task.

The baseline AR rate, on the other hand, did vary as a function of the avoidance shock strength. The mean $\pm$SE number of ARs per subject per session (including both NPS and PS sessions) was 394 $\pm$122 for subjects run at 0.15 ma avoidance shock, 732 $\pm$244 for those run at 0.5 ma, and 908 $\pm$228 for those run at 1.7 ma. A one-way analysis of variance for matched groups conducted on these data revealed a significant overall effect of avoidance shock level (F=9.8, df=2,34, $p<<.01$). Thus, although the avoidance shock level did not affect the percentage of responses which fell in each 7 1/2 min portion of the session (warmup effect) or in each type, PS or NPS, of session (prestress effect), it did affect the total number of responses made over all portions of both types of sessions.

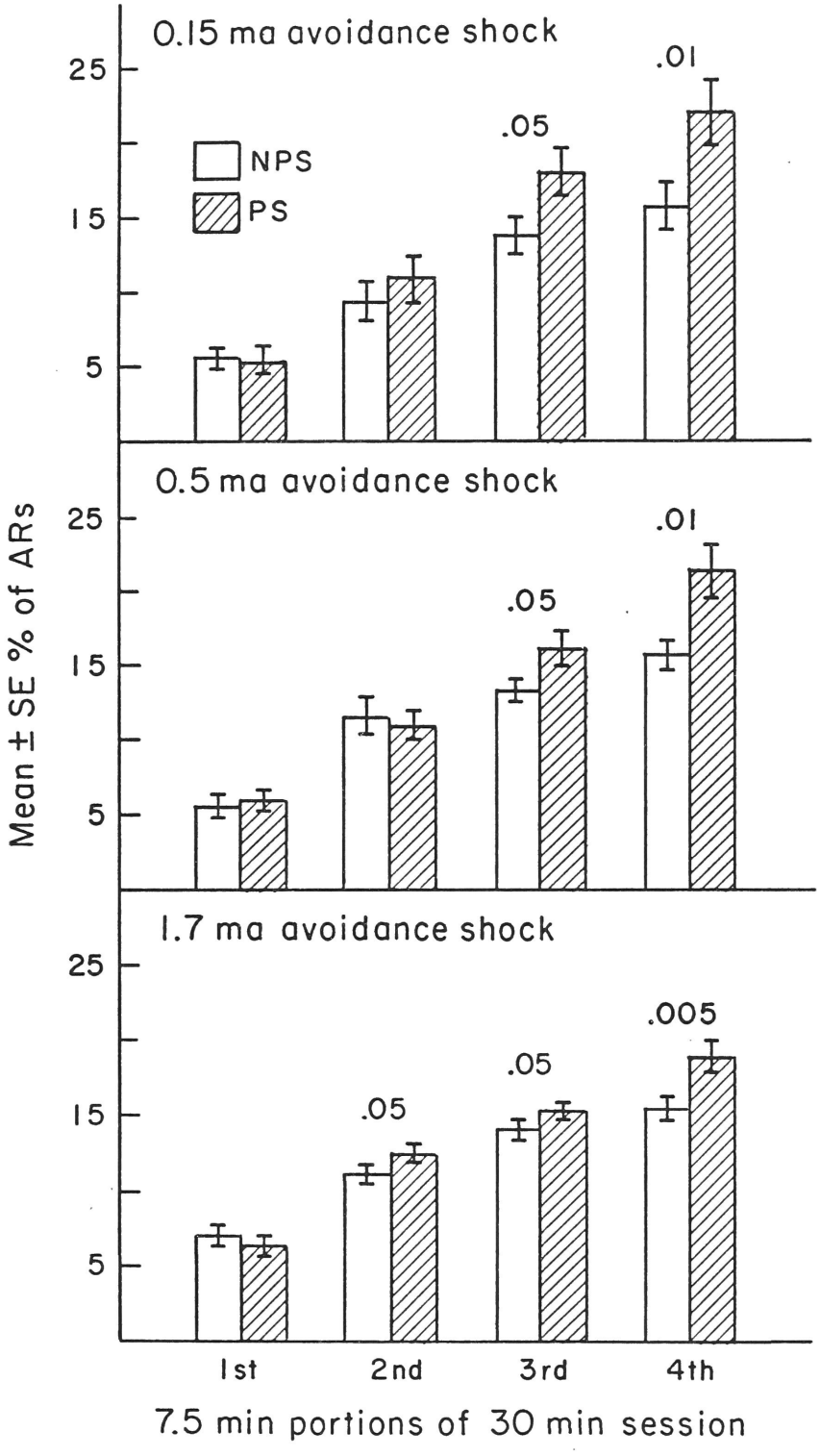

Figure 6. Mean % of ARs which occurred in each 7 1/2 min portion of NPS and PS sessions, calculated separately for the three avoidance-shock-level groups of subjects, in Expt. 5. Subjects were normal rats, and PS was foot shock delivered 30 min before the session.

The conclusion which must be drawn from this experiment is that the prestress effect is not very sensitive to the strength of electric shock used in the prestress or in the avoidance apparatus. The prestress effect occurred even when each of the two prestress shocks were much weaker than each of the many shocks received by the subject in the avoidance task itself. While increases in the avoidance shock level augmented the number of ARs subjects made, such increases did not diminish the reliability of additional augmentation caused by prestress. These results lend no support to the hypothesis, discussed in introducing this experiment, that the prestress effect is due to a rise in a central state, fear, which is affected by shock received in the avoidance box as well as by shock received in the prestress box. While fear should vary with strength of shock, the prestress effect does not.

Experiment 6

In Experiment 5, the prestress effect on avoidance responding occurred, and was not significantly changed, whether the prestress used was 0.15, 0.5 or 1.7 ma foot shock. In Experiment 2, the prestress effect occurred with air blast into the subject's home cage as the prestress. These prestresses varied greatly in severity, but all of them were observed to elicit defecation and freezing responses in the subjects, both of which are taken to be indices of fear or emotionality in the rat. The purpose of Experiment 6 was to determine whether a more innocuous prestress procedure, one which does not elicit overt signs of fear, is capable of causing the prestress effect.

A single group of nine rats were tested in the avoidance task in each of three conditions: NPS (no prestress), PS (the usual foot shock prestress, 0.5 ma), and a new condition called here "safe prestress," or SPS. The SPS was designed to be as unstressful as possible while still containing formal similarity to the PS procedure. It was like PS in that it involved removal of the subject from his home cage, placement of him into another box for 2 min, and then placement of him back to his home cage for 30 min until the avoidance session was begun. It differed from PS, however, in a number of aspects designed to minimize the fear it evoked. The most important difference was that no shock, or other intentional stressful stimulus, was given to the subject during an SPS. Another difference was that the box used for SPS was an animal cage which greatly resembled the subject's home cage, while the PS box was the usual Plexiglas chamber which greatly resembled the avoidance chamber. For example, the SPS box and the home cage both had solid floors lined with wood shavings for litter, while the PS and avoidance boxes both had grid floors and no litter. This difference was further augmented by keeping the SPS box in the animal room where the subjects lived and keeping the PS box, as previously, in the apparatus room where the avoidance sessions were held. Thus, PS presumably contained many cues similar to the avoidance procedure which would be conditioned stimuli for fear, while SPS contained many cues similar to the home cage which would be conditioned stimuli for safety.

In order to even further minimize possible fear in the SPS box, each subject was pre-adapted to the box before the experiment was begun. This was accomplished by having each subject actually live in the SPS box for a period of 9 days, ending 6 days before starting the experiment. There were three separate SPS boxes available, so the nine subjects lived in them in groups of three. Once the experiment began, the SPS box for each subject was the same cage in which he had previously lived. The experiment consisted of testing the subjects daily and employing each of the three conditions, NPS, SPS, and PS, every third day. This was continued for 18 days so that each subject was tested a total of six times in each of the three conditions. Different sequences of these conditions were assigned to different subjects in a balanced design such that each condition appeared equally often on every day of the experiment, and each condition followed every other condition equally often in the sequence. The length of the avoidance session was 30 min.

Throughout the experiment, each subject's emotional reaction to PS and SPS was quantified by counting the fecal boluses, if any, that he left in the PS or SPS box. Also, on the two days following the last day of the experiment, each subject received an additional 2 min exposure in the PS and the SPS boxes for the purpose of comparing the two boxes in elicitation of emotional responses. Five subjects were put into the SPS box on the first of these days and into the PS box on the second, and this was reversed for the other four subjects. The extra SPS exposure was like the usual SPS, but the extra PS differed from the usual one in that no shock was given. The purpose for this was to determine whether the difference in defecation observed in the two boxes was simply due to a reflexive response to electric shock, or whether it represented some other aspect of the subject's fear in the PS vs the SPS box. Also, in these final exposures, "rears" were counted as well as boluses. A rear was defined by a subject's standing on his hind paws with his forepaws either in the air or on the wall of the box. Rearing, being incompatible with freezing, was used as a measure of the absence of fear.

Results

The AR results are illustrated in Figure 7. The data were compiled in the same way as those for the previous experiments, except that the percentages were taken over three, rather than two, types of sessions with regard to prestress. It can be seen that SPS had the same augmenting effect on ARs, appearing in the second but not the first 15 min portion of the session, as did PS.

That these two prestress procedures differed markedly in their immediate effects on the overt emotionality of the subjects is shown by the defecation and rearing data in Table III. During the experiment, PS elicited defecation in every subject, while SPS did not elicit defecation in any subject. Even in the post-experiment test, with no shock used in the PS box, eight of the nine subjects defecated in the PS box, and none did in the SPS box. Likewise in the post-experiment test, only one subject made a rearing response in the PS box, while seven subjects reared in the SPS box.

It should be noted that this experiment does not eliminate the possibility that some emotional response to the prestress is necessary for the prestress effect to occur. The mere lifting up of these subjects, to put them in the SPS box, could well have been a fear-inducing experience for them, even though it did not result in defecation. The experiment does, however, indicate that a prestress below the threshold for defecation and inhibition of rearing is as effective in augmenting ARs as a prestress above that threshold.

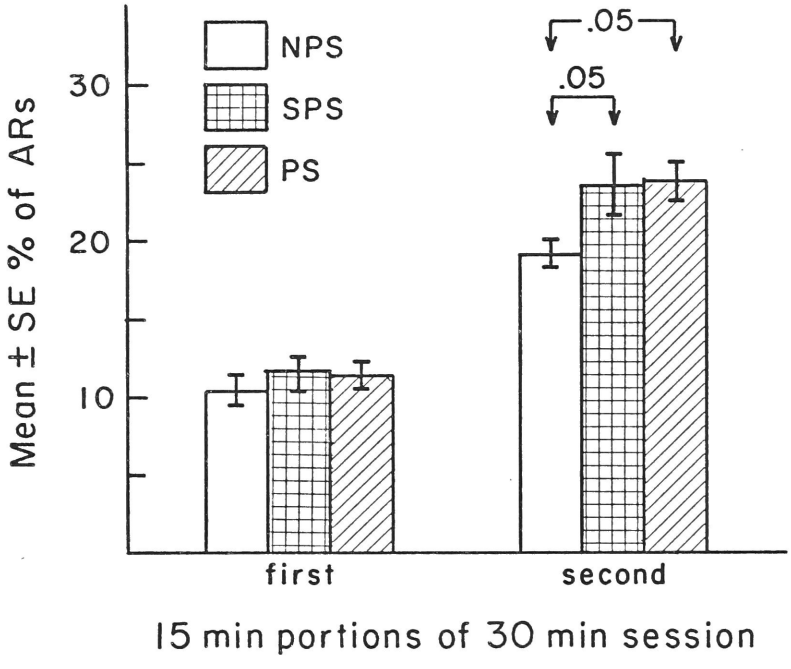

Figure 7. Mean % of ARs which occurred in each 15 min portion of NPS, SPS, and PS sessions in Expt. 6. Subjects were normal rats, SPS was exposure to a safe box 30 min before the session, and PS was foot shock delivered 30 min before the session.

Table III

Defecation and Rearing Scores for Subjects of Expt. 6 in the Safe Box and in the Prestress Box

Subject	Mean Boluses per Test During the Expt		Boluses on Single Test After the Expt		Rears on Single Test After the Expt	
	SPS	PS	SPS	PS	SPS	PS
N34	0	0.7	0	1	1	0
N35	0	4.7	0	2	2	0
N36	0	2.3	0	3	0	0
N37	0	4.0	0	3	0	1
N38	0	2.0	0	0	4	0
N39	0	3.3	0	2	7	0
N40	0	2.8	0	2	6	0
N41	0	2.8	0	3	1	0
N42	0	4.0	0	1	3	0
X	0	3.0	0	1.9	2.7	0.1

Summary

Six experiments conducted with unoperated albino rats in a particular free-operant shock-avoidance task have been described in this section. In each of these experiments, the amount of avoidance responding in a session was found to depend upon whether or not the subject was prestressed before the session. The usual procedure for prestress was to give the subject two brief electric foot shocks 30 min prior to the avoidance session, though other types of acute stressors were also used and had the same effect. Subjects who were tested daily, alternately being given prestress before one day's session and not before the next, exhibited more avoidance responding in prestressed than in non-prestressed sessions. This augmentation I have termed "the prestressed effect."

Surprisingly, the prestress effect did not appear in the initial portion of the avoidance session, but rather appeared only after a subject had been in the avoidance task for 15 min. The effect, though slow to appear, persisted to the end of the session even when, in one experiment, the session was as long as 90 min.

The prestress effect occurred, with no apparent change in reliability or magnitude, whether the subjects were tested during the "active" or "sleepy" part of their day; whether the avoidance shock current was 0.15, 0.5 or 1.7 ma; and whether the prestress was 0.15 ma shock, 0.5 ma shock, 1.7 ma shock, an air blast into the home cage, or a simple handling procedure.

HORMONAL DEPENDENCE OF THE EFFECT OF PRESTRESS DELIVERED 30 MIN BEFORE AVOIDANCE SESSIONS

The behavioral evidence with normal rats, presented in the preceding section, was encouraging for the hypothesis that stress-induced hormonal release influences avoidance responding. The time course of the prestress effect, its reversibility from day to day, and its lack of dependence upon the specific type of stressor used, were all suggestive of a hormonal process. Moreover, all of the prestresses of Experiments 1-6, including even the "safe prestress" used in Experiment 6, were probably capable of initiating release of the stress hormones ACTH, glucocorticoids, and adrenal catecholamines. Friedman, Ader, Grota, and Larson (1967), for example, found that not only electric shock, but also a variety of seemingly benign "handling" procedures caused augmented plasma corticosterone in rats.

This section of the present report describes the evidence that, while the prestress effect does depend upon the adrenal gland, it does not depend upon ACTH, glucocorticoid, or adrenal catecholamine. It further presents the evidence that no pituitary or adrenal hormone is required for the prestress effect to occur except mineralocorticoid. For all of the experiments described in this section, the parameters of the prestress (always the foot shock PS procedure) and of the avoidance task were those described in Methods. Whenever adrenalectomized rats were used, they were maintained on extra sodium chloride, as described in Methods.

Experiment 7

As the initial test of whether an adrenal hormone mediates the prestress effect, 11 adrenalectomized (adrex) rats were tested. The experimental procedure for testing the adrex rats for the prestress effect was essentially the same as that used for normal rats in Experiment 1. Each subject received one 30 min session per day, for 16 days, alternating between the foot shock PS 30 min before one day's session and NPS before the next.

Results

The results are illustrated in Figure 8. There is no sign of a prestress effect for the adrex subjects.

The lack of prestress effect in the adrex subjects could not readily be explained as due to general ill health. By weight records, and other overt signs, the subjects appeared healthy. Between the first day of training (8 days before the experiment) and the final day of the experiment, their mean ±SE weight changed from 378 ±12 g to 400 ±14 g per rat. Moreover, the subjects readily learned the avoidance task, and were not noticeably different from normal rats in their AR rates, independent of prestress. The mean ±SE number of ARs made by the adrex rats of this experiment, per subject per session (including both NPS and PS sessions), was 614 ±180. This was indistinguishable from the mean ARs per subject per session for the 44 normal rats tested at 0.5 ma shock in 30 min avoidance sessions in Experiments 1, 2, 5, and 6, which was 604 ±126. Also, as can be seen in Figure 8, the adrex subjects showed the normal warmup effect from the first to the second 15 min portion of the session.

The results of this experiment were, therefore, encouraging for the hypothesis that the prestress effect is mediated by an adrenal hormone.

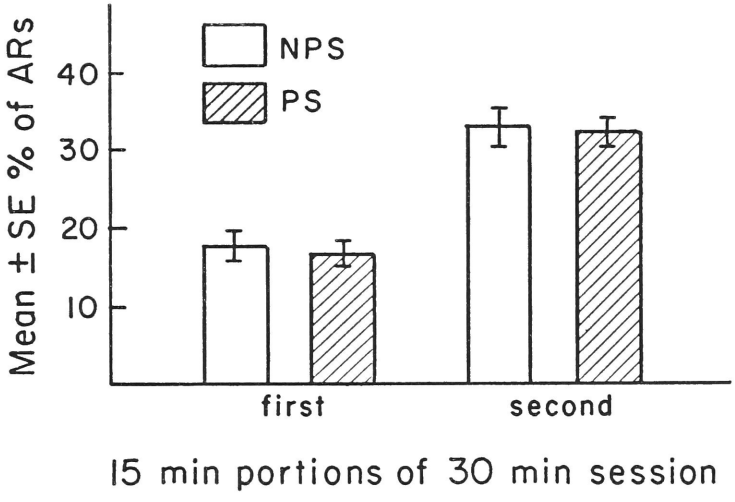

Figure 8. Mean % of ARs which occurred in each 15 min portion of NPS and PS sessions in Expt. 7. Subjects were adrex rats, and PS was foot shock delivered 30 min before the session.

Experiment 8

To determine whether glucocorticoid is the adrenal hormone essential for the prestress effect, 10 hyophysectomized (hypox) rats were tested. The synthesis of glucocorticoid in the rat adrenal is completely dependent upon ACTH, produced by the pituitary.[2] Therefore, if hypox rats showed the prestress effect, the hypothesis that the effect is mediated by glucocorticoid would have to be abandoned. The experimental procedure for testing hypox rats for the prestress effect was identical to that used for adrex rats in Experiment 7.

Results

The results are illustrated in Figure 9. The hypox subjects clearly showed the prestress effect, significant in the second, but not the first, 15 min portion of the session. Therefore, the glucocorticoid hypothesis had to be abandoned.

Besides narrowing down the possibilities concerning the mediating mechanism of the prestress effect, this experiment served as a partial control for effects of general health changes on the prestress effect. The hypox subjects appeared to be unhealthy. Although they maintained their weight when left undisturbed in their home cages before starting training for the experiment, they lost weight rapidly once training was begun. Between the first day of training (8 days before the experiment) and the final day of the experiment, their mean ±SE weight changed from 343 ±6 to 272 ±12 grams per rat. Moreover, at least in the averaged data shown in Figure 9, the hypox rats appeared abnormal in avoidance responding in that they did not show the usual warmup effect from the first to the second 15 min in NPS sessions.

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[2] A fluorimetric assay (similar to that described by Glick, von Redlich, and Levine, 1964) for plasma corticosterone in the hypox subjects of Expt. 8 was conducted, after completion of the experiment, to verify that these subjects could indeed not secrete corticosterone. The plasma was taken, by heart puncture under ether anesthesia, 30 min after an initial ether stress. No hypox rat had more than 3 µg corticosterone per 100 ml plasma (a level which could be attributed to background fluorescence) in this assay, while normal controls routinely had 25 to 35 µg per 100 ml.

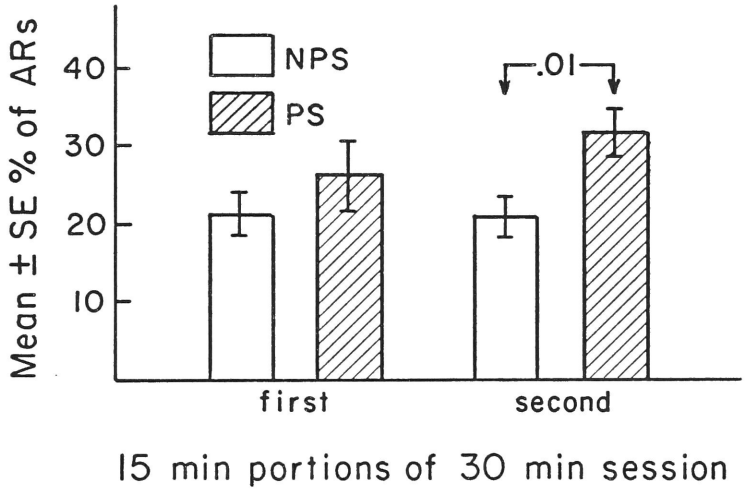

Figure 9. Mean % of ARs which occurred in each 15 min portion of NPS and PS sessions in Expt. 8. Subjects were hypox rats, and PS was foot shock delivered 30 min before the session.

In summary, although the hypox subjects were abnormal in certain respects relating to general health and to their avoidance behavior, and although they had no ACTH, glucocorticoids, or other pituitary-dependent hormones, they showed the prestress effect. In contrast to this, the adrex subjects of Experiment 7, while appearing quite normal in general health and in other aspects of their avoidance behavior, failed to show the prestress effect.

Hypothesis that the Prestress Effect Depends upon Mineralocorticoid

Both normal and hypox rats showed the prestress effect, while adrex rats did not. Since hypox rats, as well as adrex rats, lack glucocorticoids, the loss of the prestress effect in adrex rats could not be attributed to loss of glucocorticoids. One way in which an adrex rat differs from either a normal or hypox rat is that he lacks the adrenal medullae, which produce the catecholamines, epinephrine and norepinephrine. Another difference is that the adrex rat cannot produce the salt regulating steroids, mineralocorticoids, normally secreted by the adrenal cortex. Although both adrenal catecholamines and mineralocorticoids are under some control by the pituitary, their productions are not completely abolished by hypophysectomy (Wurtman and Axelrod, 1965; Palmore and Mulrow, 1969), as they are by adrenalectomy. The possibilities arose, therefore, that either adrenal catecholamines or mineralocorticoids are essential for the prestress effect to occur.

Three exploratory experiments strongly suggested that mineralocorticoid, not adrenal catecholamines, is the essential adrenal hormone for the prestress effect. Because their main conclusions are verified and extended by succeeding experiments in this report, these three experiments are only briefly summarized in the next paragraphs.

In one experiment, a group of adrenal-enucleated rats, who presumably had no adrenal medullae but had regenerated adrenal cortices, were found to show a normal prestress effect. Although it was not definitely established that the adrenals of these subjects contained no medullary tissue, this experiment provided a first indication that the adrenal medulla is not essential for the prestress effect to occur.

The second exploratory experiment indicated even more convincingly that the adrenal medulla is not essential for the prestress effect, and also suggested that mineralocorticoid is essential. A group of totally adrenalectomized rats, who initially did not show the prestress effect, did show the effect in tests conducted over a series of days

after a single injection of a long-lasting mineralocorticoid preparation, desoxycorticosterone pivalate.

In the third experiment, the influence of mineralocorticoid on the prestress effect was found to be reversible; a group of adrex rats were tested for the prestress effect both with and without treatment with desoxycorticosterone acetate (DOCA), a shorter-acting ester of desoxycorticosterone. These subjects showed the effect of prestress when treated with DOCA and lost the effect when the treatment was withdrawn.

These experiments indicated that mineralocorticoid is the only adrenal hormone required for the prestress effect to occur. They also indicated, however, that, while mineralocorticoid "permits" the effect of prestress to occur, it does not "mediate" the effect. Mediation would imply a causal sequence for the prestress effect illustrated by the following paradigm:

PS → Mineralocorticoid → Augmented ARs.

Such a causal sequence could not have occurred in the second or third exploratory studies above. The subjects in those studies, being adrenalectomized, could not secrete mineralocorticoid, and their exogenous mineralocorticoid treatment was the same for NPS sessions as for PS sessions. Therefore mineralocorticoid itself could not have caused the increment in ARs which appeared in PS compared to NPS sessions. The observed influence of mineralocorticoid is better illustrated by the following paradigm:

Mineralocorticoid present: PS → Augmented ARs.
Mineralocorticoid absent: PS → No change in ARs.

This type of influence, in which a hormone's presence is required for an effect to occur, but is not itself sufficient to cause the effect, is classically referred to as a "permissive" influence of the hormone (Ramey and Goldstein, 1957).

The hypothesis developed from these exploratory studies, and supported by all of the remaining experiments to be described in this report, is that mineralocorticoid plays an essential permissive role in the augmentation, by prestress, of avoidance behavior in the laboratory rat.

Experiment 9

The exploratory experiments, above, indicated that treatment with desoxycorticosterone renews the prestress effect in adrex rats. The purpose of Experiment 9 was to test this finding again, and to determine whether aldosterone, believed to be the main natural mineralocorticoid in the rat, also renews the effect. For this purpose, a single group of adrex rats were tested for the prestress effect under four different hormone treatment conditions including a placebo. The hormones used were the acetate esters of desoxycorticosterone (DOCA), aldosterone (Aldo-A), and corticosterone (Cort-A). Corticosterone is the main glucocorticoid of the rat (Bush, 1953), and was used in this experiment as a control substance.

Each subject was tested for the prestress effect in one hormone treatment condition for a series of eight daily sessions, alternating, as previously, between NPS and PS sessions. Then, after a single non-test day, the subject was tested for another 8 days in the same way, but with a different hormone treatment. This procedure was continued until the subject had been tested for four 8-day series, each in a different hormone treatment condition. Different sequences of hormone treatments were used for different subjects in a balanced design such that, across subjects, each of the four treatments appeared equally often in every position in the sequence, and each treatment followed every other treatment equally often in the sequence.

The hormones were injected subcutaneously, and the doses were 0.3 mg per rat per day for DOCA, 0.03 mg per rat per day for Aldo-A, and 3.0 mg per rat per day for Cort-A. The vehicle for each injection was 0.2 ml sesame oil, and this was used alone for the placebo treatment. DOCA and Aldo-A were dissolved in the oil; but Cort-A could not be dissolved at the concentration used, and therefore was suspended in the oil. The dose of Cort-A used was found in preliminary studies to result in an approximately physiological circulating corticosterone level for 24-36 hrs after administration to an adrex rat.

The injections were begun two days before the experiment began, and were continued daily until the experiment ended. In order to prevent the injection procedure itself from being a prestress, each injection was given immediately after an avoidance session, thus serving as the treatment for the next day's session. On the last day of each 8-day test series, each subject was injected with the substance to be used for the next 8-day series. Each was also injected with that substance on the non-test day between two successive test series, and thereby was maintained in the hormone condition of each test series for two days prior to the first session in the series.

The avoidance session length was 45 min, and other parameters of the task and of the prestress were the usual ones.

Results

Figure 10 illustrates the results, which were compiled, in the same way as those for previous experiments, separately for the four hormonal conditions. The subjects exhibited a significant prestress effect, in both the second and third 15 min portions of the session, when treated with either DOCA or Aldo-A, but showed no sign of an effect when treated with placebo or Cort-A.

That the mineralocorticoid treatments also differed from either of the non-mineralocorticoid treatments in a measure of mineralocorticoid activity is shown in Table IV. Consumption of tap water and of 3% NaCl solution, both of which were continuously available in the subjects' home cages, was monitored daily for each rat. As can be seen from the table, the subjects drank less saline while on either of these hormones than while on placebo or Cort-A. Cort-A had no effect, compared to placebo, on saline intake, but did have a physiological effect of another sort in that it enhanced the intake of tap water. The table also shows that the subjects gained weight during mineralocorticoid therapy and tended to stabilize in weight during placebo or Cort-A therapy. The weight gain is most likely at least partly due to mineralocorticoid-induced storage of body water.

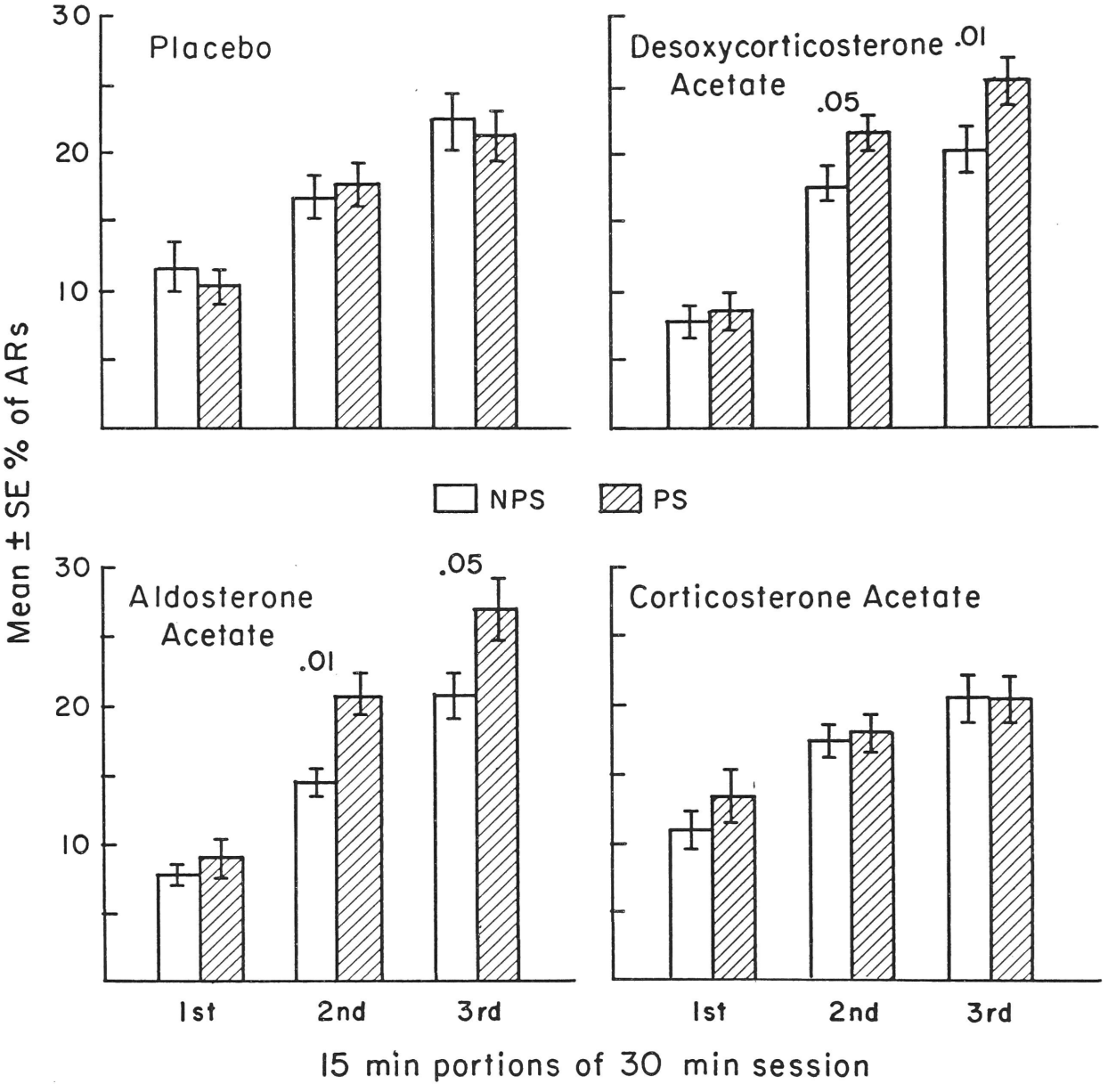

Figure 10. Mean % of ARs which occurred in each 15 min portion of NPS and PS sessions, calculated separately for four different hormone treatment conditions, in Expt. 9. Subjects were adrex rats, and PS was foot shock delivered 30 min before the session.

Table IV

Mean ±SE Body Weight Change and Fluid Intakes per Subject during 10 Days in each Hormone Treatment, Expt. 9

Treatment	Weight Change in grams	Intake of 3% NaCl in ml	Intake of Tap Water in ml
I Placebo	+2.3 ±4.0	152 ±13	423 ±27
II DOCA	+34.6 ±3.2	46 ±8	409 ±16
III Aldo-A	+24.7 ±3.6	90 ±15	383 ±17
IV Cort-A	+0.2 ±7.4	151 ±20	512 ±26

As can be seen in Table IV, Aldo-A was less effective than DOCA in reducing saline intake or in increasing body weight. But, as seen in Figure 10, it was at least as effective as DOCA in renewing the prestress effect. Although one must interpret this cautiously, it suggests that the two mineralocorticoids might have different relative potencies in their influence on the prestress effect as compared to sodium retention.

In summary, treatment with aldosterone acetate or with DOCA, both of which exhibited mineralocorticoid activity, permitted the prestress effect to occur in adrex rats, while treatment with corticosterone acetate, a glucocorticoid, did not.

Experiment 10

Treatment of adrex rats with mineralocorticoid, in Experiment 9, permitted them to show the same type of augmentation in ARs due to prestress as was previously observed with normal rats. The question arose, what is the effect of mineralocorticoid on ARs in adrex rats without prestress? It seemed possible that the permissive action of mineralocorticoid on the prestress effect, in Experiment 9, was only secondary to an effect of the hormone on the subjects' non-prestressed AR rates. The purpose of Experiment 10 was to distinguish between two hypotheses regarding the involvement of mineralocorticoid with the prestress effect.

The hypotheses are illustrated by Figure 11. According to Hypothesis 1 in the figure, the AR rate in the non-mineralocorticoid subject is abnormally high. It is at a "ceiling," such that prestress can have no further augmenting effect. One might surmise that the non-mineralocorticoid subject is essentially in a prestressed-like state, with regard to his AR rate, even when prestress has not been employed. Mineralocorticoid, by this hypotheses, restores the subject's non-prestressed rate of avoidance responding to a normal, lower, level, bringing it down from the ceiling, so that prestress can have its augmenting effect. According to Hypothesis 2, on the other hand, mineralocorticoid permits prestress to augment avoidance responding to a higher level than would be reached without mineralocorticoid.

To distinguish between these two hypotheses, certain experimental precautions had to be taken. There are large differences among different subjects in AR rate, and there are also systematic changes which occur, within subjects, in AR rate as a function of successive avoidance sessions.[3] This was the reason why, in all of the previous experiments, it was necessary to use each subject as his own control, and to alternate PS and NPS for each subject frequently within a series of avoidance tests, to see the effect of prestress on avoidance responding. The same

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[3] In general, subjects decreased their avoidance response rate as a function of successive sessions. This decrease is apparent in Figure 2 of Experiment 1.

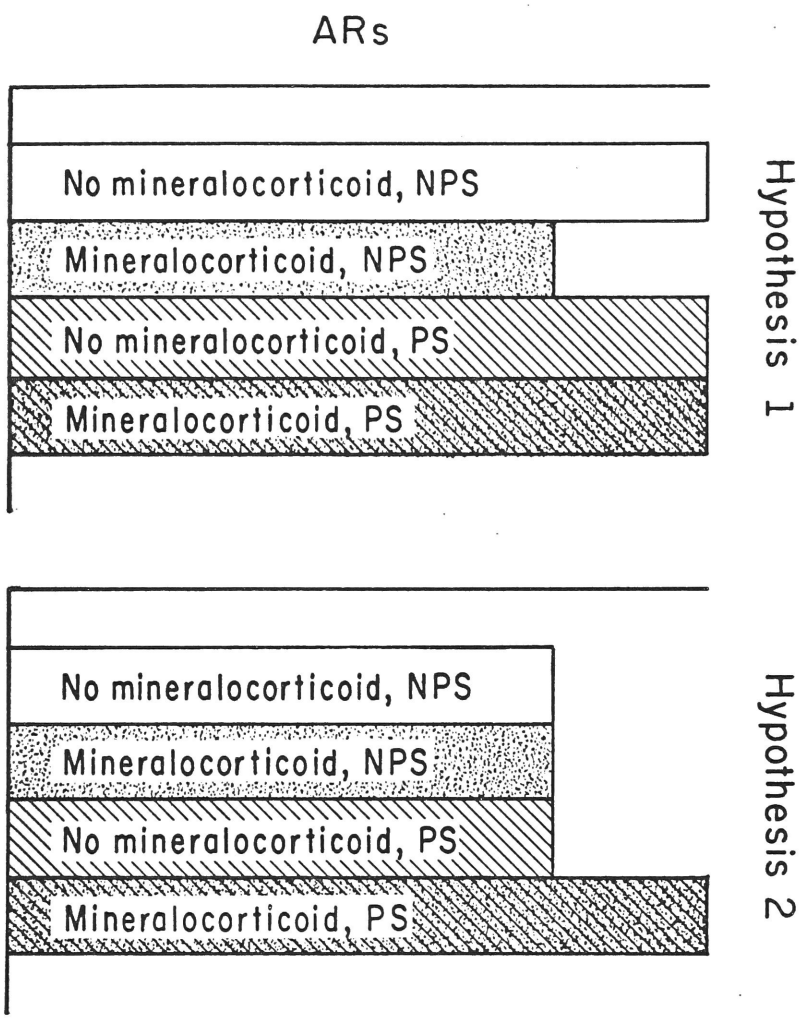

Figure 11. Two hypotheses tested in Expt. 10. By Hypothesis 1, mineralocorticoid decreases ARs made in NPS sessions. By Hypothesis 2, the hormone increases ARs made in PS sessions.

reasoning applies now as a requirement for detecting an effect of mineralocorticoid on avoidance responding: the mineralocorticoid and non-mineralocorticoid treatments must be frequently alternated in a single group of subjects over a single series of avoidance test days. In Experiment 10, therefore, to look at the effects of mineralocorticoid with and without prestress, a single group of adrex rats were tested with frequent alternation of both the hormonal and the prestress conditions.

The experiment took place over a 32-day period, and the subjects were tested only once every 2 days. The purpose for this was to allow 2 days for change from mineralocorticoid to placebo, or vice versa, between each successive session. A subject who began the experiment in the mineralocorticoid condition was injected with DOCA on each of the two days preceding his first avoidance session, and immediately after the first session he was injected with placebo; the next day, he was not tested, but was injected again with placebo; and then, on the third day, he was tested in the placebo condition and injected with DOCA immediately thereafter. This alternation was continued, for each subject, until the subject had been tested for a total of sixteen sessions in the avoidance task, eight with DOCA treatment and eight with placebo.

Besides the hormonal alternations, the PS and NPS conditions were also alternated over the series of sessions for each subject. But this alternation occurred after every two sessions rather than after every session. Thus, a subject was tested in the NPS condition twice, once with DOCA and once with placebo; then he was tested twice in the PS condition, once with DOCA and once with placebo; and so forth until the end of the experiment. In all, each subject was tested four times in each of the four combinations of hormonal and prestress conditions (DOCA, NPS; DOCA, PS; placebo, NPS; and placebo, PS). Twelve adrex rats served as subjects. Six of these started the hormonal alternation with DOCA and six started with placebo. Within each of these groups of six, three started the prestress alternation with PS and three started

with NPS.

Results

The data are illustrated in Figure 12. This figure was compiled in the same way as were previous figures showing AR data, except that the percentages were taken for four types of sessions rather than two. It is clear from the pattern shown in Figure 12 that the data support Hypothesis 2 and not Hypothesis 1. DOCA resulted in no change in the AR rate for NPS sessions, but significantly augmented the AR rate for PS sessions. Significantly more ARs were made in DOCA, PS sessions than in placebo, PS sessions, for the third 15 min portion of the session (t=2.16, df=11, p<.05), and for the combination of the second plus third 15 min portions (t=2.70, df=11, p<.05), though not for the second 15 min portion alone.

The finding that DOCA increased the number of ARs in PS sessions and had no effect on ARs in NPS sessions, indicates that mineralocorticoid is directly involved in the effect of prestress. The permissive action of mineralocorticoid on the prestress effect cannot be interpreted as a secondary result of an effect of the hormone on the subjects' AR rate independent of prestress.

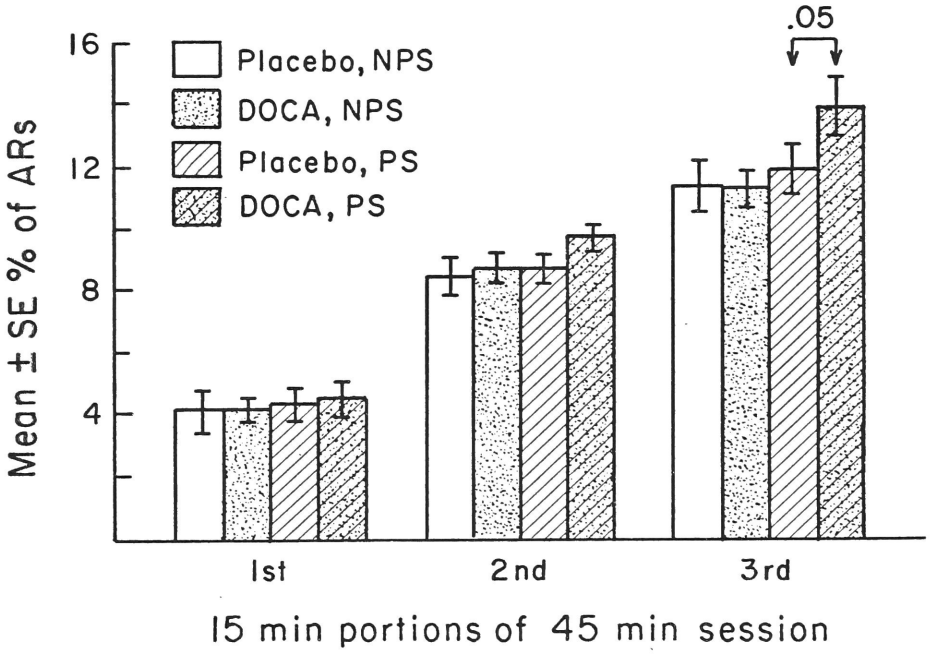

Figure 12. Mean % of ARs which occurred in each 15 min portion of four different types of sessions in Expt. 10. Subjects were adrex rats, and PS was foot shock delivered 30 min before the session. The results affirm Hypothesis 2 of Fig. 11.

Experiment 11

Does the permissive action of mineralocorticoid on the prestress effect occur only in adrex rats, or does it also occur in intact rats? To answer this, it was necessary to deplete rats of endogenous mineralocorticoid by some means other than adrenalectomy. Perhaps the most natural way to do this is to put extra sodium in their diet. Sodium results in negative feedback on aldosterone; and high sodium intake appears to completely, or nearly completely, prevent aldosterone synthesis (Bacchus, 1950; Singer, 1960). For Experiment 11, 10 unoperated rats were maintained on a saline solution as their only fluid for drinking, and were tested for the prestress effect both with and without mineralocorticoid treatment.

Each subject was tested in the avoidance task, for one session per day, for 12 days, alternating between PS for one day's session and NPS for the next. Then, after one non-test day, each subject was tested again in the same way over another series of 12 days. Five subjects received DOCA for the first 12-day series of tests and placebo for the second, and this treatment order was reversed for the other five subjects. The DOCA dose was 0.3 mg per rat per day, dissolved in 0.2 ml sesame oil, and the placebo was 0.2 ml sesame oil alone. The schedule and method for injection was identical to that for Experiment 9. The length of each avoidance session was 45 min.

The concentration of the NaCl solution on which the subjects were maintained was 2.0% for 7 days immediately preceding the experiment, and for the first 12 days of the experiment. But this was changed to 1.5% for the second 12-day series of tests due to severe weight loss by some of the subjects while on 2.0% saline. Since the subjects were counterbalanced in treatment order, they were also counterbalanced with respect to whether they were on 2.0% or 1.5% NaCl when given DOCA or when given placebo. No assays for aldosterone were performed, but it is assumed that the saline intake at least greatly reduced endogenous aldosterone production.

Results

The results are illustrated, separately for the placebo and DOCA conditions, in Figure 13. The sodium-loaded intact subjects showed no sign of a prestress effect when injected with placebo. But they did show the effect when injected with DOCA, and this was significant for the second 15 min alone (t=2.69, df=9, p<.05) and for the second plus third 15 min combined (t=2.20, df=9, p<.05), though not for the third 15 min alone.

It appears that intact rats maintained on saline, presumably thereby depleted of endogenous mineralocorticoid, show the same dependence of the prestress effect upon mineralocorticoid treatment as do adrex rats.

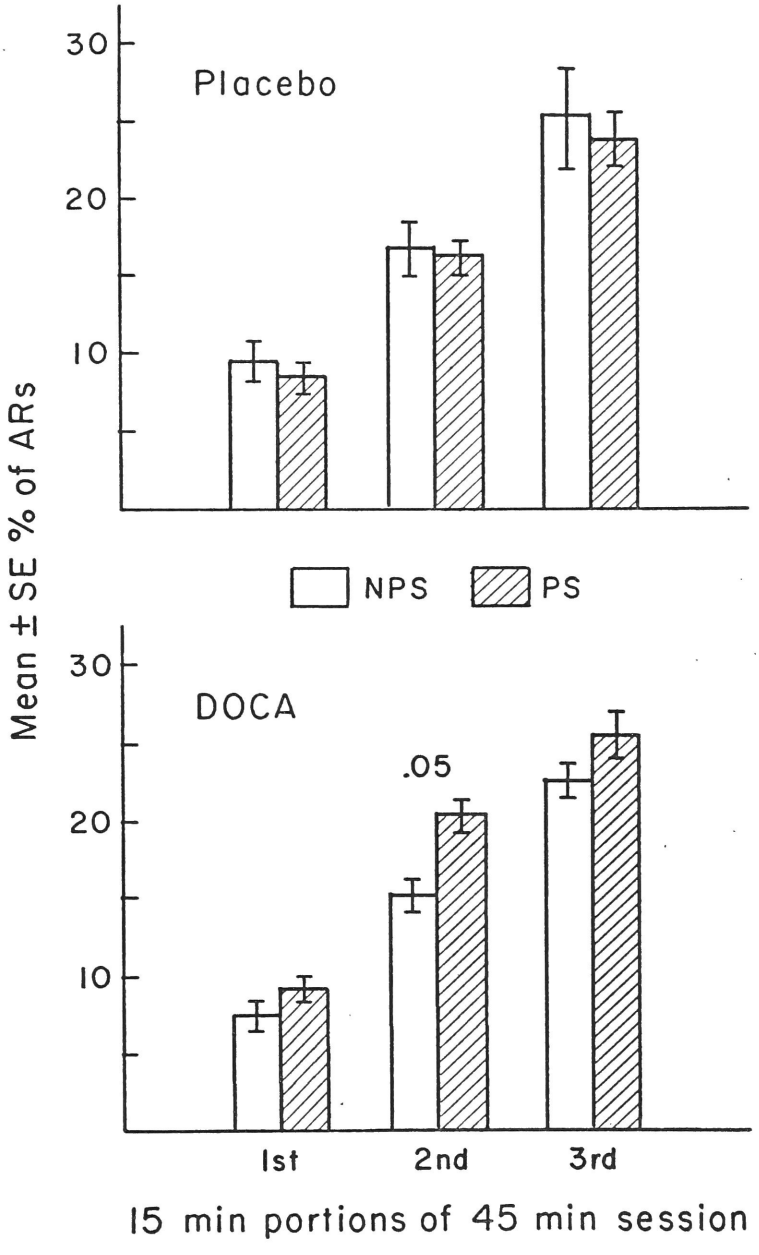

Figure 13. Mean % of ARs which occurred in each 15 min portion of NPS and PS sessions, calculated separately for placebo and DOCA treatment conditions, in Expt. 11. Subjects were sodium-loaded intact rats, and PS was foot shock delivered 30 min before the session.

Experiment 12

The purpose of Experiment 12 was to determine whether sodium-loaded intact rats, like adrex rats (in Experiment 10), show an effect of mineralocorticoid on ARs made in sessions preceded by PS and no effect in sessions preceded by NPS. Therefore, a group of saline-maintained intact rats were tested for the effect of DOCA treatment, both with and without prestress, in an experiment whose design was the same as that used for adrex rats in Experiment 10.

Twelve intact rats served as subjects, and they were maintained on 1.5% NaCl solution as their only drinking fluid for 27 days before beginning the experiment and throughout the 32 days of the experiment. In order to see possible effects of this saline maintenance, and of the DOCA treatment, on indices of general health in these subjects, their body weights and their intakes of saline and of Lab Chow were monitored throughout the experiment. Other aspects of the procedure for Experiment 12 were identical to the procedure used for Experiment 10.

Results

The results are illustrated in Figure 14. The same comparisons were made as in Experiment 10 (Fig. 12), and the same significant result was obtained. DOCA augmented ARs in PS, but not in NPS, sessions. Significantly more ARs were made in DOCA, PS sessions than in placebo, PS sessions, for the third 15 min portion of the session (t=3.17, df=11, p<.01), and for the combination of the second plus third 15 min portions (t=2.56, df=11, p<.05), though not for the second 15 min portion alone.

There was no significant effect of DOCA on saline intake, Lab Chow intake, or body weight changes in these subjects. The mean ±SE 2-day intake of 1.5% NaCl was 162 ±34 ml per rat with placebo treatment, and 166 ±39 with DOCA treatment. The same for intake of Lab Chow was 43 ±1.5 g with placebo, and 44.4 ±1.4 g with DOCA. And the mean ±SE change in body weight per rat was +2.9 ±1.4 g with placebo, and +0.2 ±1.6 g with DOCA.

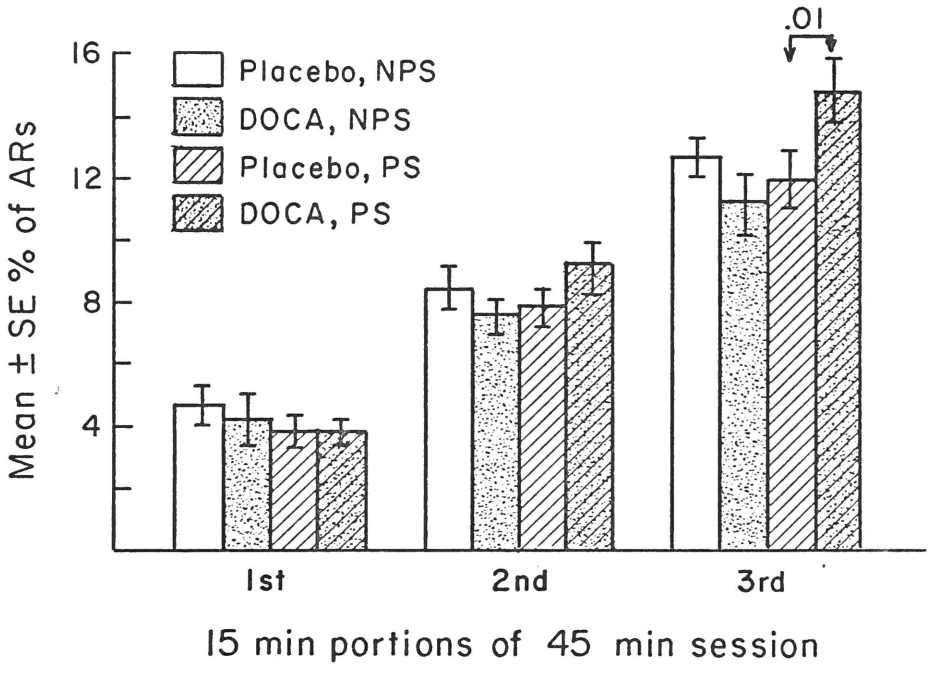

Figure 14. Mean % of ARs which occurred in each 15 min portion of four different types of sessions in Expt. 12. Subjects were sodium-loaded intact rats, and PS was foot shock delivered 30 min before the session.

In conclusion, unoperated rats maintained on 1.5% NaCl solution as their only fluid for drinking were like adrex rats (of Expt. 10) in that DOCA significantly increased the number of ARs they made in PS sessions and had no effect on ARs in NPS sessions. The behavioral effect of DOCA was not secondary to an effect on NaCl intake, Lab Chow intake, or body weight of the subjects, as there was no effect of DOCA on these measures.

Summary

The prestress effect, which in previous experiments (Expts. 1-6) had been found to persist in normal rats under a wide variety of environmental conditions, was found in the present section to disappear if the rats were adrenalectomized. The effect did not, however, disappear if the rats were hypophysectomized. And the effect reappeared in adrex rats if they were treated with mineralocorticoid, either DOCA or aldosterone acetate.

The prestress effect also disappeared if intact rats were maintained on a saline solution as their only source of fluid for drinking, a procedure which presumably inhibited endogenous mineralocorticoid production. And the effect reappeared in intact saline-maintained rats if they were treated with DOCA.

With either adrex or saline-maintained intact rats, DOCA injection augmented avoidance in sessions preceded by prestress and had no significant effect in sessions not preceded by prestress.

The action of mineralocorticoid on the prestress effect was identified as a permissive one rather than a mediating one. It was not necessary for the rat to secrete mineralocorticoid in response to the prestress to show the prestress effect. Adrex rats, who could not secrete the hormone, and who had a constant level of the hormone for NPS and PS sessions due to injection, nevertheless showed the prestress effect. The presence of mineralocorticoid, not its secretion in response to stress, is required for prestress to have its effect on avoidance responding.

EFFECT OF PRESTRESS IN NORMAL AND ADRENALECTOMIZED RATS AS A FUNCTION OF TIME BETWEEN PRESTRESS AND THE AVOIDANCE SESSION

A rat who lacks mineralocorticoid is behaviorally different from a rat who does not lack mineralocorticoid in that his avoidance response rate is not augmented by prestress given 30 min before the avoidance session. What, more specifically, is the nature of the behavioral deficit in the mineralocorticoid-depleted subject? Are there any conditions under which the rat can show a prestress-induced augmentation in avoidance responding without mineralocorticoid?

In all of the preceding experiments, a delay of 30 min, spent by the subject in his home cage, succeeded prestress and preceded the avoidance session. One requirement of the subject, therefore, if he was to show the prestress effect, was that he retain some consequence of the prestress over a 30 min period in his home cage. The possibility arose, therefore, that the deficit in the mineralocorticoid-depleted rat lies not in his immediate response to the prestress, but rather is an inability to retain the response, or some crucial aspect of the response, for 30 min in his home cage. This possibility was investigated in the remaining three experiments by looking at the effects of prestresses delivered less than 30 min before the avoidance session. Except for the prestress-to-avoidance interval, all other parameters of the avoidance task, and of the prestress, were kept constant, and were as described in Methods.

Experiment 13

The purpose of Experiment 13 was to determine whether shortening the prestress-to-avoidance interval (delay), to less than 30 min, would in any way change the effect of prestress on avoidance responding in normal rats. For this purpose, four different conditions of delay were used. One was the usual 30 min delay, the second was a 5 min delay, and the third was a 1 min delay. For each of these three delays, the subject was returned to his home cage before starting an avoidance session. For the fourth delay condition, designated "no delay," the subject was not returned to the home cage before starting the avoidance session, but rather spent one extra min in the prestress box (no shock was given during the extra min) and then was placed directly into the avoidance box. By keeping the subject in the prestress box for an extra min, the fourth condition was equated with the third (1 min delay) condition in the length of time between initiation of the prestress and initiation of the avoidance session.[4] The four delay conditions are shown graphically in Figure 15.

Sixteen normal rats served as subjects, and each subject was tested for the effect of prestress with each of the four delays. A given subject was first tested with one delay, in a series of eight daily sessions alternating between NPS for one day's session and PS, with the appropriate delay, for the next. Then, after a single non-test day, the subject was tested for another series of 8 days, in the same way, but with a different delay following PS. This procedure was continued until each subject had been tested for four separate 8-day series, each series using a different one of the four delays. Different sequences of the delay conditions were used for different subjects in a balanced design, such that, across subjects, each condition appeared

[4] The 1 min delay, in the third condition, included the time required to return the subject to the animal room, after the PS, and to bring him back to the apparatus room, for the avoidance session. Thus, only 20 to 30 sec of the 1 min delay was actually spent, by the subject, undisturbed inside his insulated box in the animal room.

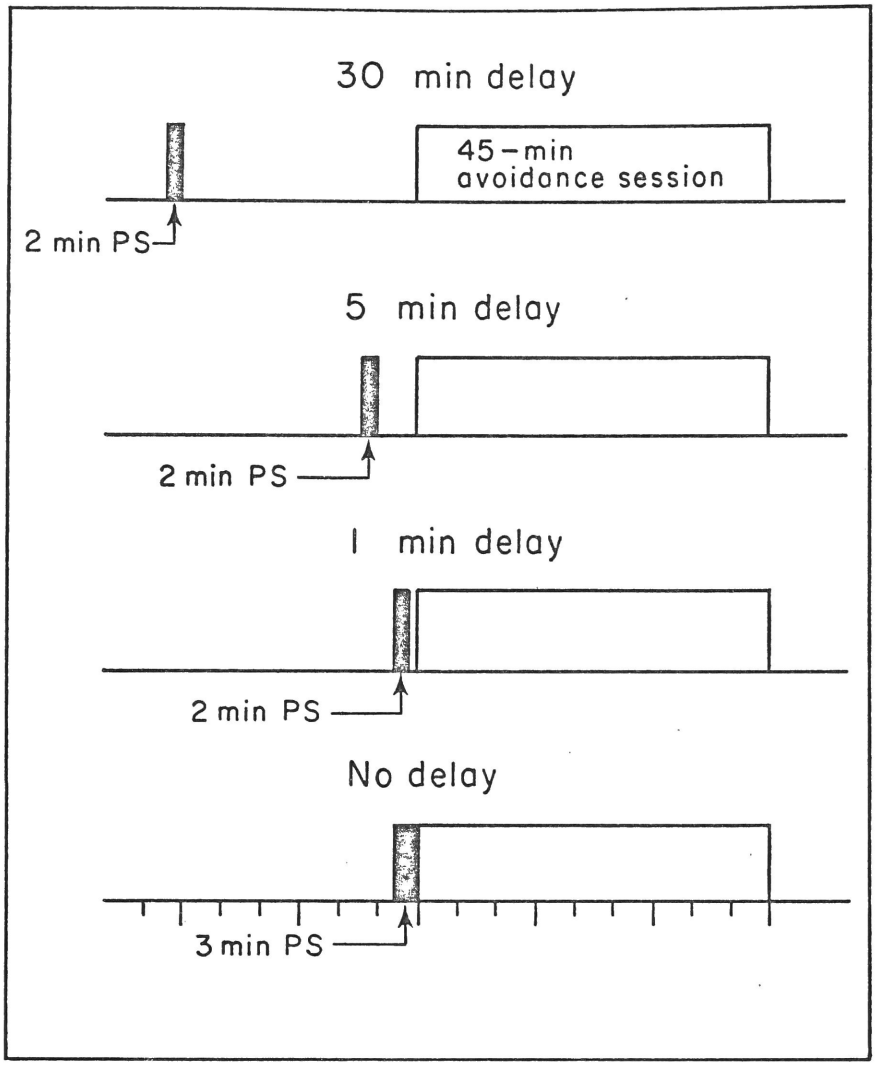

Figure 15. The four delay conditions used in Expt. 13. In each condition except that of "no delay," the delay was spent, by the subject, in his home cage.

equally often in every position in the sequence, and each condition followed every other condition equally often in the sequence. The length of the avoidance session was 45 min.

Results

The results are illustrated, separately for each of the four delay conditions, in Figure 16. Prestress significantly augmented ARs in each delay condition except that of no delay. The effect of prestress with 1 min or 5 min delay appeared to be different, however, from the effect with 30 min delay. With either 1 min or 5 min delay in the home cage, PS significantly augmented ARs in all three 15 min portions of the session! In contrast, PS with 30 min delay, for these subjects as well as for those of all preceding experiments, augmented ARs only after the first 15 min portion of the session.

The delay between prestress and initiation of the avoidance session apparently plays an important role in determining the type of effect prestress has on avoidance responding.

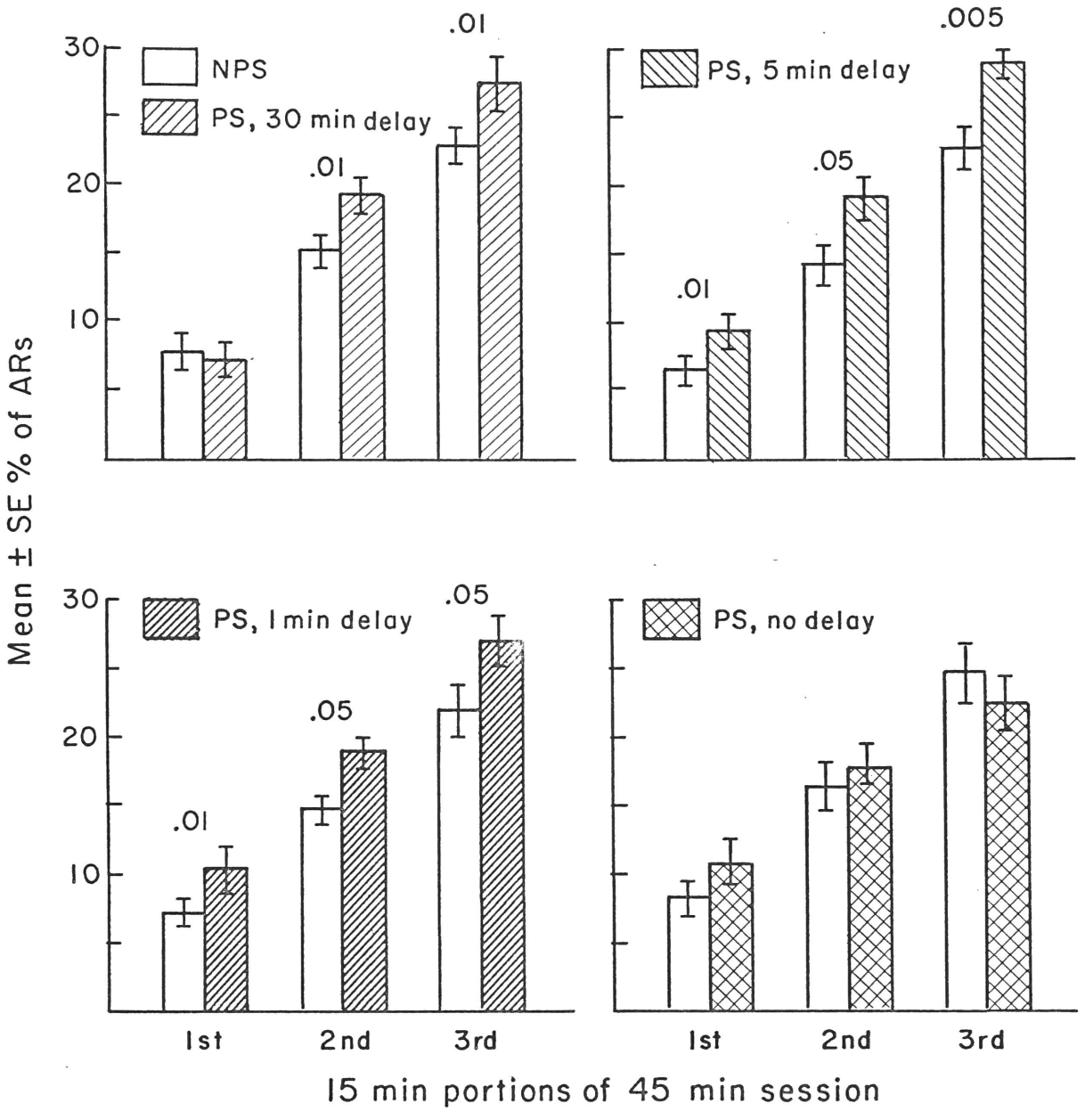

Figure 16. Mean % of ARs which occurred in each 15 min portion of NPS and PS sessions, calculated separately for four different delay conditions, in Expt. 13. Subjects were intact rats, PS was foot shock, and the delays were as shown in Fig. 15.

Experiment 14

Does the effect of prestress with 1 min delay in the home cage, like the effect with 30 min delay, depend upon mineralocorticoid? To answer this question, a group of adrex rats were tested for the effect of prestress with 1 min delay.

An additional purpose of Experiment 14 was to test again the observation, made in Experiment 13, that the effect of prestress in normal rats varies as a function of the prestress-to-avoidance delay.

For these purposes, eight adrex and eight normal rats served as subjects. Two delay conditions were employed: no delay and 1 min delay. These were precisely the same conditions as the fourth and third conditions, respectively, of Experiment 13 (see Fig. 15). In order to directly compare the results of the two different delays with each other, as well as with NPS, each subject was tested with each of the two delays in every third session, and with NPS in the remaining third session, of the series of avoidance sessions which constituted the experiment. Different sequences of the alternation, among NPS, PS with no delay, and PS with 1 min delay, were employed, for different subjects, in a design which was balanced, as nearly as possible, for their order in the sequence. The experiment was conducted over an 18-day period, with each subject receiving one avoidance session per day. The length of the avoidance session was 45 min.

Results

The results are illustrated, separately for the two types of subjects, in Figure 17. Most importantly, it can be seen that PS with 1 min delay augmented ARs in not only the normal subjects, but also the adrex subjects! The effect of PS with 1 min delay, compared to NPS, was significant for every 15 min portion of the session for adrex rats. The effect of PS with 1 min delay, compared to NPS, was also significant for the third 15 min portion for normal rats, but only approached significance ($.05<p<.10$) for each of the first and second 15 min portions. PS with no delay seemed to have a smaller effect, both for normal and

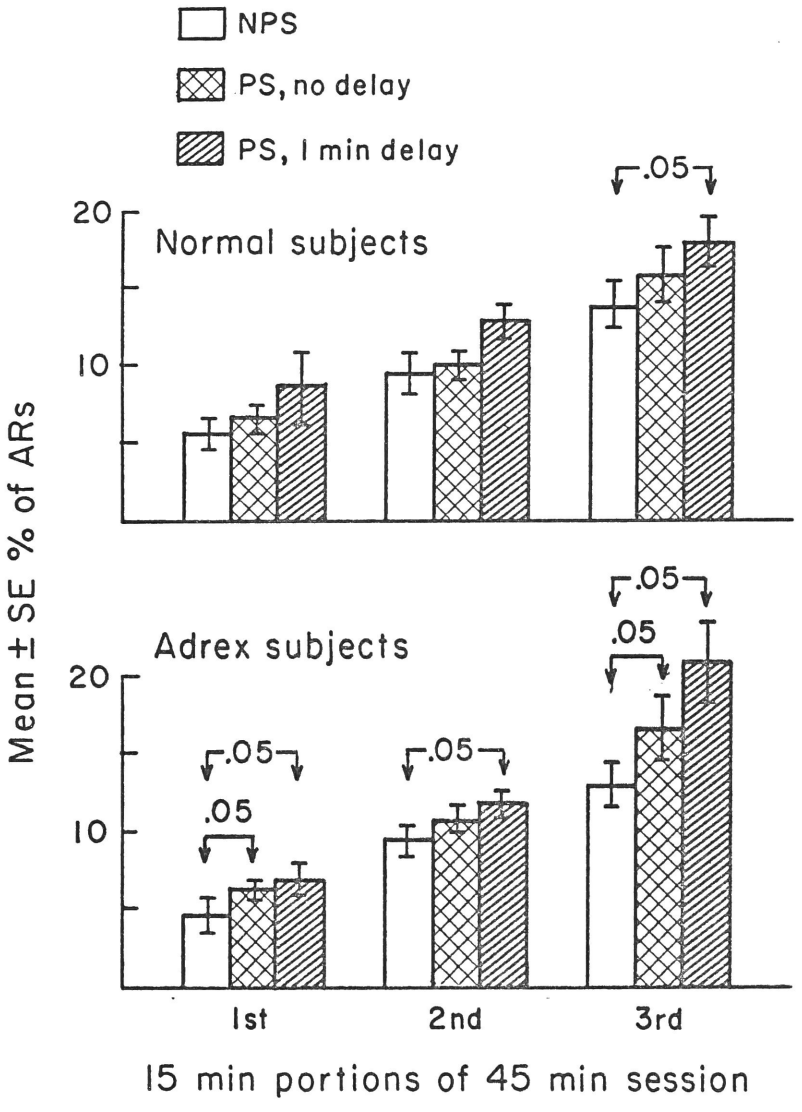

Figure 17. Mean % of ARs which occurred in each 15 min portion of NPS and two types of PS sessions, calculated separately for normal and adrex subjects, in Expt. 14.

adrex rats, than did PS with 1 min delay. More ARs were made in sessions preceded by PS and no delay than in sessions preceded by NPS, by both types of subjects and in all three 15 min portions of the session, but this effect reached significance only for the adrex subjects, third 15 min portion.

In conclusion, prestress increased the number of ARs made by untreated adrex rats when the delay between prestress and the avoidance session was 1 min. This was in contrast to the repeated lack of any effect of prestress in untreated adrex rats, in previous experiments, when the delay was 30 min. The effect of PS with 1 min delay differs, apparently, from the effect with 30 min delay, in two regards: it appears in the first 15 min portion of the session as well as later, and it does not require the presence of mineralocorticoid.

Experiment 15

The purpose of Experiment 15 was to directly test the differences between PS with 1 min delay and PS with 30 min delay. Eight normal and eight adrex rats served as subjects, and they were tested in the avoidance task in each of three conditions: NPS, PS with 1 min delay, and PS with 30 min delay. The two delay conditions corresponded, respectively, with the third and first conditions of Experiment 13 (see Fig. 15). Except for use of the 30 min delay condition instead of the no delay condition, the procedure was the same as that used for Experiment 14.

Results

The results, illustrated in Figure 18, confirmed the findings of the previous experiments. In adrex rats, PS with 1 min delay increased ARs, significantly in the first and third 15 min portions of the session, while PS with 30 min delay had no effect. In normal rats, PS with 1 min delay increased ARs in all three 15 min portions of the session, though, in this experiment, this effect did not reach significance ($.05<\underline{p}<.10$ for each 15 min portion or for all portions combined). And, once again, PS with 30 min delay increased ARs for normal rats in the second and third portions of the session, but not the first.

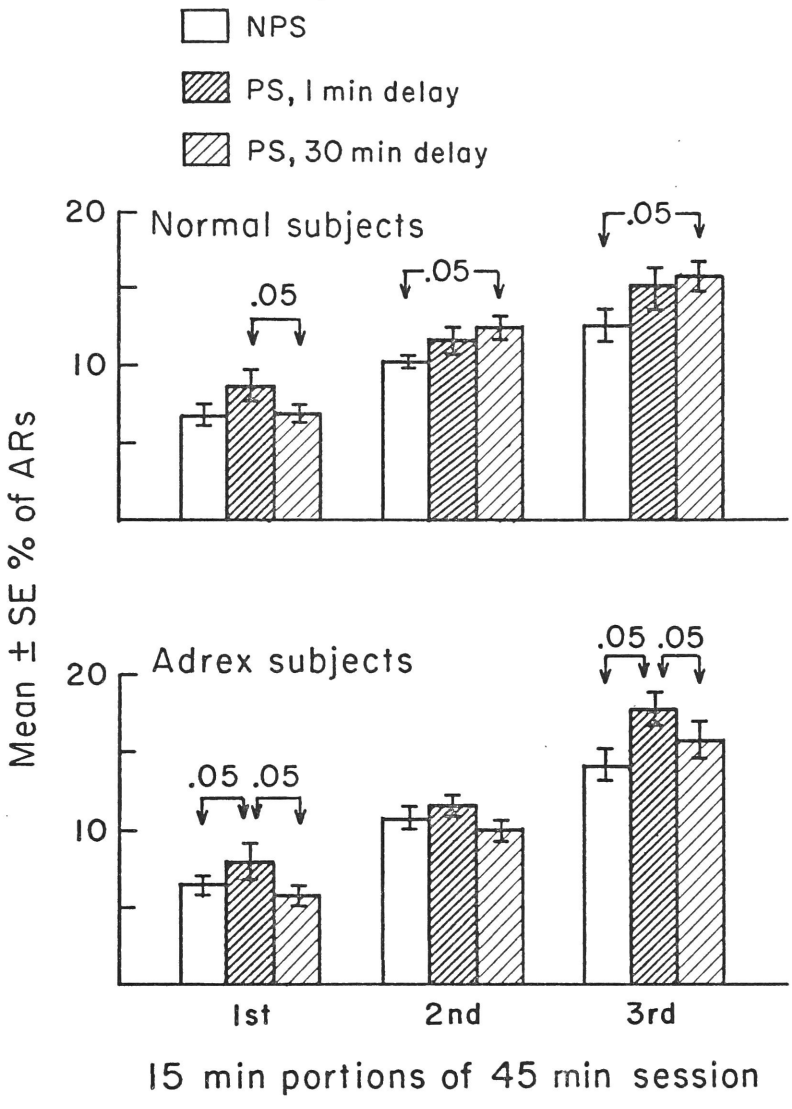

Figure 18. Mean % of ARs which occurred in each 15 min portion of NPS and two types of PS sessions, calculated separately for normal and adrex subjects, in Expt. 15.

Summary

Shortening the delay between prestress and the avoidance session changed the effect of prestress on avoidance responding. While prestress followed by 30 min delay in the home cage increased ARs only after the first 15 min portion of the session, the same prestress followed by 1 min (or 5 min) in the home cage increased ARs in all portions of the session. The effect with 1 min delay also differed from the effect with 30 min delay in that it occurred in adrex rats who were not treated with mineralocorticoid.

If no delay in the home cage followed prestress, but the rat spent 1 extra min in the prestress box before being placed directly into the avoidance chamber, a prestress effect may have occurred for both normal and adrex rats, but it was less reliable than the effect with 1 min delay in the home cage.

DISCUSSION

The importance of the experiments described in this report lies in the findings of (a) a prolonged augmenting effect on avoidance behavior, within a session, of prestress delivered shortly before the session begins, and (b) a reliable and specific permissive influence of mineralocorticoid on a non-appetative behavioral phenomenon.

The Prestress Effect as a Behavioral Phenomenon in Normal Rats

Although most psychologists are aware that stressing subjects shortly before behavioral testing may influence the results of the test, this has generally been regarded as a point of procedural control rather than a matter for study in itself. An exception is a study by Posluns and Vanderwolf (1970), who found that the acquisition of one-way avoidance was facilitated in rats if they were exposed to the avoidance chamber, or to another unfamiliar box, for 5 min immediately preceding the training session. They found no facilitation if the same type of exposure was given 24 hrs before the training session. This effect seems similar to the prestress effect described in the present report.

In the present study, any of a variety of acute stressors, delivered to rats as prestress 30 min prior to an avoidance session, enhanced avoidance responding in the session. This effect occurred independently of whether the stressor was a very benign one, as was the safe prestress of Experiment 6, or a very severe one, as was the 1.7 ma foot shock prestress of Experiment 5. Similarly, the effect did not appear to depend upon the degree of resemblence between the stimulation used for the prestress and that used for the avoidance task. An air jet into the subject's home cage (Expt. 2) which greatly differed from the avoidance stimulation, had the same effect as the usual foot shock prestress, which was highly similar to the avoidance stimulation. Moreover, the effect of prestress was as reliable the first time a prestress was used with a group of rats as it was after a number of uses, implying

on the one hand, that a build-up of association between prestress and the avoidance task was not necessary, and, on the other, that the prestress effect was not subject to habituation with repeated trials. Taking these characteristics together, the prestress effect appears to be the result of some reversible physiological state, which is temporarily imposed on the subject by environmental disturbance. The prestress effect does not seem to require that the subject experience a great amount of fear, or emotionality, in response to the prestress, and it does not seem to involve a learning process.

While the effect on avoidance responding did not vary as a function of the type of prestress used, it did vary as a function of the delay between prestress and the avoidance session. As shown in the final three experiments of this report, prestress followed by 1 min (or 5 min) in the home cage results in a different within-session pattern of avoidance responding than did prestress followed by 30 min in the home cage. These different patterns are described, below, each in relation to the pattern shown in control, non-prestressed, sessions.

With no prestress, subjects began the session responding at a very low rate, and as the session progressed their response rate increased. This is the warmup effect. If prestress was given, and was followed by only 1 min (or 5 min) delay in the home cage, the subjects began the session responding at a higher rate than they would have without prestress, and still continued to warm up so that their rate remained higher, even at the end of the session, than it would have been without prestress. Thus, the prestress effect with 1 min delay might well be considered a "pre-warmup effect." Prestress with 1 min delay may have served the same function as did time in the avoidance apparatus itself, and thereby initiated the warmup process before the session was begun. Prestress with 30 min delay in the home cage, on the other hand, had an effect which could not be considered a pre-warmup effect. With 30 min delay after prestress, the subjects began the avoidance session responding at the same low rate as they would have without prestress. With this

condition of delay, the subjects made more avoidance responses in the later portions of the avoidance session than they would have without prestress, but not in the initial portion. Thus, the prestress effect with 30 min delay may be described as a predisposing of the subjects to show a greater warmup effect, than they would without prestress, after they enter the avoidance apparatus.

Dependence of the Prestress Effect upon Mineralocorticoid

As indicated in the introduction to this report, an important reason for starting the study of the effect of prestress on avoidance behavior was to determine whether any pituitary or adrenal hormone mediates the effect. The results, that hypophysectomized (Expt. 8) and mineralocorticoid-treated adrenalectomized (Expts. 9 and 10) rats both showed the prestress effect, prove that the effect is not mediated by the release of any pituitary or adrenal hormone. The effect does depend upon mineralocorticoid, but it is the presence of a basal level of mineralocorticoid, not the release of the hormone in response to stress, which is essential. This point was already made (see section following Expt. 8) in describing the mineralocorticoid influence as a "permissive," rather than "mediating," one.

These results should not be taken to mean that stress-released pituitary and adrenal hormones have no influence on avoidance behavior. But they do indicate that whatever influence the hormones do have must not appear in all avoidance situations or measures. That the effects of ACTH and glucocorticoids on avoidance are subtle ones, appearing in some avoidance situations and not others, has already been suggested by Weiss, McEwen, Silva and Kalkut (1969).

The present finding, of an essential permissive role of mineralocorticoid on a stress-induced enhancement of avoidance, could not have been predicted from the experimental literature. The only repeatedly-studied effects of mineralocorticoid are those directly related to salt appetite (e.g. Fregly and Waters, 1965; Wolf, 1964). There is very

little documentation of any non-appetative behavioral effects of mineralocorticoid. An exception is a report that desoxycorticosterone treatment in intact rats decreases their mouse-killing behavior (Kostowski, Rewerski and Piechocki, 1970), but that effect has no obvious parallels to the effect observed in the present study.

The behavioral influence of mineralocorticoids in the present study was a very specific one. This specificity can be illustrated by considering the several observed similarities, and the single observed difference, between adrenalectomized and normal rats in avoidance behavior. The two types of subjects were similar in their baseline avoidance response rates without prestress, they were similar in the within-session warmup effect, and they were similar in the effect of prestress completed 1 min before the avoidance session. The only difference was in the effect of prestress completed 30 min before the avoidance session. Normal rats showed the effect, while adrenalectomized rats did not show the effect unless they were treated with mineralocorticoid. The mineralocorticoid treatment did not influence the rate of avoidance responding in non-prestressed sessions, and it did not influence the warmup effect. The only observed behavioral effect of the treatment was to permit the effect of prestress, with 30 min delay, to occur.

How, physiologically, mineralocorticoid acts to permit the prestress effect is unknown. But the behavioral results provide at least a clue. As was pointed out in the first part of this Discussion, prestress followed by 1 min delay in the home cage had a different effect on avoidance responding in normal rats than did prestress followed by 30 min delay. This must mean that prestress with 1 min delay imposes some physiological state on the subject which causes him to behave in a certain way once he enters the avoidance task, and that this physiological state changes to a different one with further time (30 min) in the home cage. It is the development, with time, of the second state, not the first, which depends upon mineralocorticoids. Apparently, some delayed physiological reaction to stress can occur if mineralocorticoid

is available, but cannot occur if mineralocorticoid is not available.

The most well-known physiological effect of mineralocorticoid is to conserve body sodium, by acting on the kidney and other epithelial tissues. This effect of the hormone, on overall body sodium level, however, does not appear to be the mechanism by which mineralocorticoid permitted the prestress effect to occur. Intact sodium-loaded rats (Expts. 11 and 12), as well as adrenalectomized rats, required exogenous mineralocorticoid to show the prestress effect. Overall body sodium in the sodium-loaded subjects was presumably already abnormally high, and mineralocorticoid injection would presumably raise their body sodium to an even more abnormally high level; yet the injection returned the subjects to normal with regard to the prestress effect.

Mineralocorticoid has other physiological effects, however, besides retention of overall body sodium, and some of these effects might well be normalizing for sodium-loaded intact, as well as adrenalectomized, rats. Mineralocorticoid promotes the excretion, at the kidney, of potassium, hydrogen, ammonium, and magnesium (Mulrow, 1967). It also affects the distribution, within the body, of sodium and other ions. Aldosterone has been found to retard sodium exchange in dog erythrocytes (Spach and Streeten, 1964), to decrease muscle sodium relative to serum sodium in adrenalectomized rats (French and Manery, 1963), and to increase the ratios of extracellular to intracellular sodium and of intracellular to extracellular potassium in the brain and muscle of mice (Woodbury and Koch, 1957). Specific binding proteins for aldosterone have been found in many tissues, including brain (Swaneck, Highland and Edelman, 1969). Mineralocorticoid has also been found to have a suppressing effect on epileptic seizures in man and electroshock seizures in rats (Woodbury, 1958). These various physiological effects of mineralocorticoid suggest many ways by which the hormone could potentially influence behavior, but they provide no clue as to why the hormone should have had the specific type of behavioral influence which was observed in the present study.

Whatever physiological effect it is of mineralocorticoid which permits the prestress effect to occur, it must be one which occurs at low circulating levels of the hormone. Normally-maintained intact rats showed the prestress effect. So did normally-maintained hypophysectomized rats; and hypophysectomized rats have been reported to secrete less mineralocorticoid than intact rats. Palmore and Mulrow (1967) found that hypophysectomized rats, maintained on a diet which was normal in sodium content, secreted only about 1/4 as much aldosterone as did intact rats similarly maintained. If this difference in aldosterone secretion, between hypox and normal rats, was true for the subjects in the present study, it must mean either that hypox rats require less mineralocorticoid to show the prestress effect than do intact rats, or that as little as 1/4 normal secretion rate is sufficient for intact rats to show the effect. The latter possibility is not inconsistent with the finding that saline maintenance abolished the prestress effect in intact rats. Although aldosterone secretion was not measured in these subjects, previous literature indicates that the 1.5% NaCl maintenance condition, employed in Experiments 11 and 12, may well have decreased aldosterone output to less than 1/4 normal. Singer (1960) found that maintaining rats on 0.9% NaCl, as their only drinking fluid for one week, reduced their aldosterone output to about 1/3 that of normally-maintained controls. And Bacchus (1950) found that rats maintained on 2.5% NaCl, as their only drinking fluid for 9 days or more, showed signs of structural and functional atrophy of the zona glomerulosa (the adrenal cortical zone which produces aldosterone).

Although the level of mineralocorticoid necessary for the prestress effect to be abolished is very low, it is a level which is not incompatible with health in intact rats on high sodium diet. In Experiment 12 of the present study, intact rats who were maintained on 1.5% NaCl for 59 days, and who did not show the prestress effect without exogenous mineralocorticoid, exhibited no weight loss or other apparent signs of ill health. In fact, other investigators have found that intact rats choose to drink more saline, in concentrations as high as

1.3% (Bare, 1949), than tap water when given continuous access to both. It seems possible, therefore, that the behavioral phenomenon represented by the prestress effect can appear and disappear, in the normal rat, as a function of the rat's own choice of diet.

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APPENDIX

The avoidance response (AR) data for the individual subjects of all 15 experiments are given in the following tables. The first data column in each table is the total of the mean number of ARs made by the subject in all 15 min portions of all types of sessions employed in the experiment, and the remaining columns give the percentage of that total which fell in each portion of each type of session. The *t*s shown at the bottom of the tables represent the results of dependent *t* tests. The significance levels (*p*s) associated with these *t*s are the same as those presented in the figures for the experiments.

Experiments 1, 2 and 3

Rat	ARs per NPS+PS pair of sessions	% of ARs			
		1st 15 min		2nd 15 min	
		NPS	PS	NPS	PS
Experiment 1					
N1	362	16.3	12.4	25.4	45.9
N2	629	12.4	11.8	34.0	41.8
N3	938	7.1	17.3	26.3	49.3
N4	4720	16.9	22.2	28.7	32.2
N5	5094	15.1	14.6	32.6	37.7
N6	503	13.7	10.3	39.2	36.8
N7	573	6.5	13.8	33.2	46.6
X:		12.6	14.6	31.3	41.5
SE:		1.6	1.5	1.8	2.3
				t = 2.91	
				p < .05	
Experiment 2					
N14	258	10.7	14.8	35.7	38.8
N15	293	17.6	15.3	35.4	31.7
N16	136	20.8	15.4	28.1	35.7
N17	175	20.8	21.6	19.5	38.2
N18	984	26.1	17.6	30.6	25.7
N19	161	13.6	17.8	30.4	38.3
N20	107	23.0	34.2	15.2	27.7
N21	490	20.3	12.0	36.7	31.1
N22	99	22.2	19.2	25.6	33.0
N23	296	15.8	5.7	32.4	46.0
X:		19.1	17.4	29.0	34.6
SE:		1.5	2.3	2.3	1.9
				t = 2.32	
				p < .05	
Experiment 3					
N14	480	5.8	8.8	29.7	55.7
N15	417	10.8	3.4	34.8	51.0
N16	257	11.8	24.9	33.5	29.7
N17	258	10.9	23.9	30.2	35.0
N18	1199	16.8	21.7	26.4	35.0
N19	137	17.8	19.2	13.4	49.7
N20	232	18.0	16.4	28.6	37.0
N21	380	27.4	24.3	27.9	20.4
N22	310	17.9	17.1	32.2	23.8
N23	205	18.2	34.4	19.6	27.8
X:		15.5	19.4	27.6	37.4
SE:		1.9	2.8	2.1	3.6
				t = 2.15	
				p < .05	

Experiment 4

Rat	ARs per NPS+PS pair of sessions	Percent of ARs											
		1st 15 min		2nd 15 min		3rd 15 min		4th 15 min		5th 15 min		6th 15 min	
		NPS	PS	NPS	PS	NPS	PS	NPS	PS	NPS	PS	NPS	PS
#1	1049	3.6	2.5	5.4	4.8	5.9	6.6	6.9	12.7	9.8	11.1	13.0	17.7
#2	749	4.9	5.7	7.0	11.4	7.5	11.3	5.8	11.7	6.8	10.5	7.4	9.9
#3	4183	2.1	2.8	6.0	6.7	8.1	7.4	10.7	11.3	8.2	13.3	8.8	14.6
#4	846	2.1	2.1	5.2	7.8	10.0	11.3	7.8	9.4	8.5	13.2	11.4	11.2
#5	570	1.9	1.5	4.5	7.8	3.0	10.7	7.0	13.0	10.0	14.0	12.1	15.2
#6	626	6.1	4.4.	8.5	12.5	8.3	12.5	7.8	10.5	6.1	9.4	5.4	8.5
#7	2347	0.9	1.4	4.7	6.4	8.2	8.8	8.9	10.0	10.1	12.6	11.4	16.7
#8	8340	4.7	5.6	8.5	8.4	9.8	9.8	8.9	9.4	8.9	8.9	8.7	9.2
#9	8303	4.0	4.7	5.1	5.4	7.1	7.3	8.7	10.3	9.8	11.4	12.0	14.2
#10	1009	1.7	1.4	3.1	7.1	6.4	10.9	8.9	13.1	10.5	13.5	11.0	12.3
X:		3.20	3.21	5.80	7.77	7.43	9.59	8.14	11.14	8.87	11.79	10.12	12.95
SE:		0.54	0.55	0.55	0.75	0.64	0.64	0.44	0.45	0.47	0.57	0.77	1.01
				$t = 3.39$		$t = 2.48$		$t = 4.18$		$t = 5.79$		$t = 4.47$	
				$p < .005$		$p < .05$		$p < .005$		$p < .001$		$p < .001$	

Experiment 5

PS level	Rat	ARs per NPS+PS pair of sessions	Percent of ARs: 1st 7 1/2 min NPS	1st 7 1/2 min PS	2nd 7 1/2 min NPS	2nd 7 1/2 min PS	3rd 7 1/2 min NPS	3rd 7 1/2 min PS	4th 7 1/2 min NPS	4th 7 1/2 min PS
0.15 ma Avoidance Level										
	LL1	4276	8.7	7.6	11.8	9.2	16.4	14.9	16.2	15.1
	LL2	854	4.2	2.6	12.9	11.6	21.4	15.0	16.1	18.5
0.15	LL3	134	0.7	7.9	3.0	4.7	18.0	24.7	16.2	24.7
ma	LL4	258	5.6	2.3	5.7	10.9	4.8	14.5	22.1	34.2
	LL5	440	6.2	5.2	8.1	10.1	11.8	16.9	20.3	21.6
	LL6	316	12.0	15.2	13.1	10.5	11.2	11.6	8.8	17.4
	ML1	2043	4.2	3.6	13.0	14.0	12.4	22.4	12.5	17.8
	ML2	1598	7.2	8.8	15.5	13.8	14.6	11.8	9.9	18.2
0.50	ML3	776	4.7	6.3	8.4	9.9	13.3	18.5	19.1	20.0
ma	ML4	561	2.3	1.8	6.3	9.1	14.8	22.6	23.5	19.6
	ML5	257	2.7	2.6	2.5	7.7	14.1	14.8	8.4	47.0
	ML6	45	2.5	0.9	7.6	0.9	20.3	18.4	22.2	27.0
	HL1	1194	9.5	5.9	21.2	9.5	23.9	13.9	8.1	8.0
	HL2	614	3.6	1.1	9.3	6.9	11.9	21.1	22.4	23.8
1.70	HL3	464	2.6	3.1	4.2	9.5	9.5	18.7	17.3	35.2
ma	HL4	246	13.6	3.4	15.3	6.8	12.6	10.0	18.5	19.8
	HL5	78	4.0	11.9	9.2	13.4	10.8	16.7	18.9	15.0
	HL6	23	5.1	5.1	0.0	35.5	5.7	34.8	1.2	12.6
	X:		5.5	5.3	9.3	10.8	13.7	17.8	15.6	22.0
	SE:		0.8	0.9	13.	1.6	1.1	1.4	1.5	2.2
							t = 2.02		t = 2.70	
							p < .05		p < .01	

0.50 ma Avoidance Level

	LM1	8906	13.5	11.0	13.1	13.1	13.2	11.3	11.4	13.4
	LM2	1052	8.6	3.2	11.1	12.2	14.0	11.9	18.7	20.2
0.15	LM3	910	4.1	9.8	14.2	13.1	18.8	16.4	9.8	13.8
ma	LM4	314	2.5	4.8	7.8	3.1	15.3	19.0	20.9	26.4
	LM5	709	4.2	4.7	8.5	13.0	11.8	14.5	21.0	22.3
	LM6	7.3	1.9	1.9	4.0	4.0	7.8	31.4	19.6	29.4
	MM1	2079	10.0	3.5	22.1	15.9	14.1	9.9	15.1	9.3
	MM2	1871	5.9	6.8	13.5	12.6	15.1	14.0	15.8	16.3
0.50	MM3	940	3.0	1.8	6.4	12.3	8.7	17.5	15.4	34.9
ma	MM4	651	1.7	5.4	3.6	8.1	15.4	18.7	18.6	28.4
	MM5	969	3.6	5.8	5.8	10.2	9.8	20.2	18.5	25.7
	MM6	165	7.3	6.9	11.7	8.3	12.7	16.0	13.1	24.1
	HM1	3896	6.6	10.1	14.5	13.7	13.9	14.3	12.6	14.4
	HM2	1648	8.5	5.9	19.2	13.8	13.8	14.1	15.5	9.2
1.70	HM3	877	2.8	3.7	11.0	13.1	13.8	19.7	13.7	22.3
ma	HM4	759	2.6	4.2	9.2	9.4	14.4	17.7	16.6	26.1
	HM5	193	5.9	6.9	21.4	6.0	13.3	9.3	8.3	29.0
	HM6	406	8.4	8.6	10.3	13.9	13.0	13.3	15.7	16.8
	X:		5.6	5.8	11.5	10.9	13.3	16.1	15.6	21.2
	SE:		0.8	0.6	1.3	0.9	0.6	1.2	0.9	1.7
							t = 1.80		t = 3.30	
							p < .05		p < .005	

1.70 ma Avoidance Level

	LH1	9142	10.4	9.3	14.1	12.8	14.4	12.9	14.0	12.2
	LH2	1202	3.9	4.3	10.2	12.6	14.0	15.9	17.6	21.4
0.15	LH3	1744	6.6	5.0	12.3	13.0	16.3	14.6	16.1	16.1
ma	LH4	1170	14.2	8.8	5.5	7.1	12.3	12.5	20.5	31.6
	LH5	785	2.2	3.6	4.5	9.5	11.7	16.2	20.5	31.6
	LH6	882	7.8	9.8	11.1	11.8	13.0	14.3	16.9	18.3
	MH1	2457	5.7	7.6	13.7	12.9	18.1	15.5	11.6	14.8
	MH2	2639	8.2	5.2	11.3	11.5	13.2	15.5	16.3	18.8
0.50	MH3	920	3.5	3.0	8.9	13.2	14.4	18.2	17.6	21.1
ma	MH4	1871	7.3	5.3	14.1	11.1	16.7	14.6	13.7	17.3
	MH5	1389	5.3	5.1	11.9	16.8	15.2	14.3	10.6	20.8
	MH6	813	4.6	5.9	12.3	12.9	16.3	17.4	14.0	16.6
	HH1	2337	9.7	9.5	13.2	12.6	12.5	13.3	13.9	15.4
	HH2	1227	8.1	6.7	11.8	14.3	12.7	13.7	13.4	19.1
1.70	HH3	1762	6.2	7.8	10.6	12.9	11.4	14.1	12.5	24.5
ma	HH4	436	7.3	8.4	10.6	10.3	17.1	16.6	15.0	14.7
	HH5	1528	7.7	6.3	11.5	11.8	12.4	16.9	15.2	18.3
	HH6	396	5.3	6.5	11.7	14.1	11.9	16.2	16.2	18.1
	X:		6.9	6.4	11.1	12.3	14.1	15.2	15.4	18.7
	SE:		0.7	0.5	0.6	0.5	0.5	0.4	0.7	1.0
					t = 2.38		t = 1.87		t = 3.50	
					p < .05		p < .05		p < .005	

Experiment 6

Rat	ARs per NPS+SPS+PS triplet of sessions	Percent of ARs					
		1st 15 min			2nd 15 min		
		NPS	SPS	PS	NPS	SPS	PS
SB1	3634	11.6	9.9	13.1	22.6	22.7	20.0
SB2	508	9.6	14.1	12.6	15.5	21.4	26.7
SB3	2914	13.8	14.8	14.1	19.7	20.3	17.2
SB4	1179	5.1	7.7	8.2	16.9	33.8	28.3
SB5	250	11.9	13.5	12.8	16.5	15.5	29.7
SB6	1556	10.2	12.6	12.4	19.1	24.3	21.2
SB7	752	9.6	10.2	10.8	20.1	25.2	24.2
SB8	1610	9.8	9.8	6.8	21.4	29.0	23.2
SB9	4190	12.3	12.7	10.4	21.1	20.4	23.2
X:		10.4	11.7	11.2	19.2	23.6	23.7
SE:		0.8	0.8	0.8	0.8	1.8	1.3

$t = 2.33$
$p < .05$

$t = 2.27$
$p < .05$

Experiments 7 and 8

Rat / Rat	ARs per NPS+PS pair of sessions	Percent of ARs: 1st 15 min NPS	1st 15 min PS	2nd 15 min NPS	2nd 15 min PS
Experiment 7 (adrex rats)					
Ax2	340	14.8	21.3	29.9	33.9
Ax3	822	15.8	17.8	33.5	32.9
Ax4	428	17.3	15.9	32.8	34.0
Ax5	903	35.7	12.7	32.1	19.9
Ax6	1461	11.9	20.3	31.2	36.6
Ax7	241	19.1	18.0	32.6	30.3
Ax8	1693	19.5	20.7	22.4	37.4
Ax9	392	14.3	16.4	31.3	38.0
Ax10	1956	16.5	17.3	32.4	33.8
Ax11	905	16.3	9.1	50.9	23.8
Ax12	4382	16.0	13.1	36.2	34.7
X:		17.9	16.6	33.2	32.3
SE:		1.9	1.1	2.1	1.7
Experiment 8 (hypox rats)					
Hx1	198	10.7	3.9	33.9	51.5
Hx2	1496	30.7	24.4	26.0	18.8
Hx3	1246	25.1	48.9	7.8	18.2
Hx4	98	13.4	46.5	18.7	21.3
Hx5	870	13.4	21.6	24.3	40.7
Hx6	1653	14.5	22.2	19.8	43.6
Hx7	33	20.1	36.2	13.1	30.7
Hx8	1920	23.0	20.3	27.3	29.4
Hx9	389	40.5	12.4	13.3	33.8
Hx10	611	21.4	25.3	23.2	30.2
X:		21.3	26.2	20.7	31.8
SE:		2.9	4.5	2.5	3.5

$t = 3.53$

$p < .01$

Experiment 9

Rat	ARs per NPS+PS pair of sessions	Percent of ARs: 1st 15 min NPS	1st 15 min PS	2nd 15 min NPS	2nd 15 min PS	3rd 15 min NPS	3rd 15 min PS
Placebo Treatment Condition							
Ax21	3445	12.0	14.4	15.1	19.7	19.4	19.5
Ax22(1)	265	15.7	13.6	8.2	19.0	19.9	23.6
Ax23(4)	3215	12.3	8.3	21.1	15.5	21.1	21.6
Ax24(1)	241	5.7	6.3	11.4	14.2	35.1	26.9
Ax25(3)	21	22.9	12.0	9.6	13.3	24.1	18.1
Ax26(4)	331	15.2	12.6	21.2	12.3	19.0	19.7
Ax27(3)	317	12.6	15.8	16.8	18.8	18.5	17.8
Ax28(3)	61	25.8	19.7	13.1	24.6	8.2	8.6
Ax29(2)	876	9.0	10.3	21.5	22.2	19.5	17.5
Ax30(4)	615	1.8	0.2	6.5	4.5	44.6	42.5
Ax31(4)	652	2.0	1.5	27.1	34.1	20.3	15.2
Ax32(3)	671	6.3	11.0	19.7	21.5	15.7	25.9
Ax33(1)	2240	12.6	11.6	16.3	20.1	17.0	22.5
Ax34(2)	807	14.3	5.3	27.0	14.7	27.0	14.2
Ax35(1)	289	5.7	7.5	17.2	11.9	31.3	26.5
Ax36(2)	8183	13.3	15.0	17.5	17.3	18.2	18.8
X:		11.7	10.3	16.8	17.7	22.3	21.2
SE:		1.7	1.3	1.5	1.6	2.1	1.9
DOCA Treatment Condition							
Ax21(4)	1177	8.7	8.2	12.6	24.9	14.1	31.5
Ax22(2)	99	5.3	25.2	18.7	27.2	5.3	18.2
Ax23(3)	2835	6.3	11.2	16.8	20.9	20.9	14.0
Ax24(3)	71	3.5	2.8	19.7	21.4	19.0	29.6
Ax25(1)	1275	8.7	13.0	15.5	23.3	16.3	23.2
Ax26(2)	268	5.1	7.8	5.2	10.8	25.8	45.1
Ax27(4)	36	18.2	7.7	15.4	32.9	9.1	16.8
Ax28(4)	37	5.2	2.0	29.3	22.4	19.1	21.4
Ax29(1)	1324	12.4	9.2	22.7	16.7	17.3	21.7
Ax30(2)	3868	5.9	6.6	15.2	22.5	23.1	26.7
Ax31(3)	1101	5.6	2.4	18.7	21.4	29.4	22.5
Ax32(1)	1162	7.4	7.7	19.0	23.1	20.2	22.5
Ax33(2)	2042	11.2	11.4	15.8	18.8	18.9	23.9
Ax34(1)	2355	5.5	7.2	23.1	17.3	22.6	24.3
Ax35(3)	54	3.2	0.9	16.1	15.2	36.4	28.1
Ax36(4)	7307	11.6	12.1	17.8	18.1	20.3	20.1
X:		7.8	8.5	17.6	21.2	19.9	25.0
SE:		1.0	1.5	1.3	1.3	1.8	1.7
				$t = 2.09$		$t = 2.73$	
				$p < .05$		$p < .01$	

Experiment 9 (continued)

Aldosterone Acetate Treatment Condition

Ax21(1)	4321	14.1	12.4	17.1	19.1	16.6	20.7
Ax22(3)	71	12.3	14.8	9.9	16.9	20.8	25.4
Ax23(1)	7142	13.0	13.7	16.2	22.3	17.6	17.2
Ax24(4)	29	6.1	6.1	19.2	20.0	18.3	28.7
Ax25(2)	162	10.2	13.4	8.8	36.3	16.2	15.0
Ax26(1)	337	8.5	9.8	21.6	15.1	25.4	19.7
Ax27(2)	570	6.3	5.8	13.6	29.7	13.4	31.2
Ax28(1)	691	7.6	3.8	11.1	11.2	12.8	53.5
Ax29(4)	411	6.0	8.2	15.9	16.1	27.5	26.4
Ax30(3)	2244	4.1	6.3	10.3	17.1	30.6	31.6
Ax31(2)	872	6.3	4.1	16.5	23.2	20.7	29.2
Ax32(4)	604	7.0	6.1	11.0	28.3	21.1	26.6
Ax33(4)	1124	2.7	2.6	17.3	14.7	35.7	27.0
Ax34(3)	380	7.6	23.9	15.3	22.3	10.5	20.4
Ax35(2)	89	4.8	12.0	12.4	22.3	24.2	34.4
Ax36(3)	7042	10.7	12.3	16.0	18.6	19.2	23.1
X:		8.0	9.1	14.5	20.8	20.7	26.9
SE:		0.8	1.4	0.9	1.6	1.7	2.2
				t = 3.00		t = 2.19	
				p .005		p .05	

Corticosterone Acetate Treatment Condition

Ax21(3)	2002	8.0	12.8	17.4	10.0	20.1	22.5
Ax22(4)	67	5.6	16.1	23.6	13.5	21.3	19.8
Ax23(2)	3100	11.1	6.6	25.5	13.3	25.2	19.4
Ax24(2)	134	6.9	1.0	22.6	15.0	17.3	25.0
Ax25(4)	132	10.6	29.5	14.6	26.0	10.6	8.6
Ax26(3)	302	14.1	14.5	19.0	16.8	10.7	24.8
Ax27(1)	724	6.4	4.5	20.2	28.6	11.2	19.0
Ax28(2)	101	15.8	31.1	12.1	23.2	11.9	5.9
Ax29(3)	754	10.0	12.3	17.5	19.3	20.9	20.1
Ax30(1)	3307	10.2	9.1	19.4	18.6	21.6	21.2
Ax31(1)	1387	7.8	7.3	13.8	19.1	25.9	26.1
Ax32(2)	1470	7.1	9.0	17.8	20.5	23.2	22.5
Ax33(3)	2068	5.9	14.7	9.1	21.4	19.7	29.2
Ax34(4)	358	24.1	10.3	11.5	11.5	30.0	13.1
Ax35(4)	18	14.3	17.1	11.4	11.4	27.1	18.6
Ax36(1)	11957	14.4	13.7	18.9	17.2	19.1	16.7
X:		10.9	13.2	17.3	17.9	20.4	20.3
SE:		1.2	2.0	1.2	1.3	1.6	1.7

*The number (1,2,3, or 4) in parentheses after each subject indicates the position in the sequence of treatments at which the subject received the hormone indicated by the table.

Experiment 10

Rat	ARs per *O,NPS+D,NPS +O,PS+D,PS quadruplet of sessions	Percent of ARs											
		1st 15 min				2nd 15 min				3rd 15 min			
		NPS		PS		NPS		PS		NPS		PS	
		O	D	O	D	O	D	O	D	O	D	O	D
Ax41	43	1.7	2.3	1.7	1.7	6.4	9.2	5.8	8.7	14.5	11.0	16.2	20.8
Ax42	3721	2.2	1.9	2.2	2.0	5.9	8.3	8.5	10.0	13.2	14.3	16.9	14.7
Ax43	2406	3.5	3.0	3.4	5.3	6.8	10.0	9.8	10.0	10.1	10.7	12.9	14.5
Ax44	1803	4.0	4.0	4.4	6.0	6.9	7.7	7.3	8.1	15.2	10.8	12.6	13.1
Ax45	2638	2.2	3.8	2.4	2.4	8.4	11.5	8.9	10.5	11.9	15.9	12.3	9.9
Ax46	5329	9.3	5.1	5.9	3.9	8.7	4.9	9.5	9.8	9.3	10.6	11.6	11.5
Ax47	1315	2.6	2.5	2.4	3.0	8.6	6.6	7.1	10.7	14.0	11.4	10.9	20.2
Ax48	7307	5.0	4.0	6.8	7.2	11.3	9.4	10.8	10.1	8.4	8.3	8.6	10.2
Ax49	2270	8.1	3.8	3.8	4.4	11.2	8.5	7.9	10.0	10.2	8.7	10.4	13.1
Ax50	1416	2.9	6.5	6.6	6.6	8.4	8.3	11.1	7.7	7.3	12.2	7.5	15.0
Ax51	2368	2.5	3.1	3.3	3.1	9.8	10.4	8.0	8.1	15.1	13.4	12.2	11.0
Ax52	4044	5.0	5.9	7.4	7.3	8.1	8.8	9.0	10.6	7.1	9.1	9.9	11.8
X:		4.1	3.8	4.2	4.4	8.4	8.6	8.6	9.5	11.4	11.4	11.8	13.8
SE:		0.7	0.4	0.6	0.6	0.5	0.5	0.5	0.5	0.8	0.7	0.8	1.0
								t = 1.67				t = 2.16	
								n.s.				p < .05	

*O = Placebo
D = DOCA

Experiment 11

Rat	ARs per NPS+PS pair of sessions	Percent of ARs: 1st 15 min NPS	1st 15 min PS	2nd 15 min NPS	2nd 15 min PS	3rd 15 min NPS	3rd 15 min PS
Placebo Treatment Condition							
SS1	704	7.5	11.6	16.1	18.1	24.2	22.6
SS2	842	4.2	8.0	14.6	21.7	21.2	30.4
SS3	847	17.6	4.3	23.6	17.5	22.4	14.6
SS4	882	8.6	7.7	19.8	16.5	31.5	16.0
SS5	1007	11.7	12.5	19.1	20.3	19.4	17.0
SS6	309	3.9	2.6	6.7	9.8	52.1	24.8
SS7	2535	8.7	9.6	14.6	18.0	23.2	25.9
SS8	648	10.0	12.0	16.5	16.1	16.1	29.4
SS9	15	11.1	7.8	12.2	15.3	27.8	25.5
SS10	35	11.1	9.1	23.7	10.6	15.2	30.3
X:		9.4	8.5	16.7	16.4	25.3	23.7
SE:		1.3	1.0	1.6	1.2	3.4	1.9
DOCA Treatment Condition							
SS1	461	9.3	12.0	14.0	19.0	19.6	26.2
SS2	1498	6.0	11.9	17.8	18.9	20.4	25.0
SS3	451	10.4	9.7	9.5	27.4	22.5	20.4
SS4	1347	7.8	11.8	20.6	19.4	22.1	18.4
SS5	8800	11.4	10.0	19.5	19.3	20.4	19.3
SS6	1521	7.0	6.5	13.9	19.5	25.8	27.2
SS7	1052	5.3	7.4	13.8	16.2	23.3	34.1
SS8	1084	9.3	9.7	13.6	22.7	17.3	27.4
SS9	1299	3.9	5.5	15.7	19.2	27.2	28.5
SS10	69	4.3	6.8	15.0	18.8	26.2	28.5
X:		7.5	9.1	15.3	20.4	22.5	25.5
SE:		0.8	0.8	1.0	1.0	1.0	1.5
				$t = 2.69$		$t = 1.53$	
				$p < .05$		n.s.	

(Note. Subjects SS2, SS4, SS6, SS7, and SS10 received placebo first, and the other five received DOCA first.)

Experiment 12

Rat	Ars per *O,NPS+D,NPS +O,PS+D,PS Quadruplet of sessions	Percent of ARs											
		1st 15 min				2nd 15 min				3rd 15 min			
		NPS		PS		NPS		PS		NPS		PS	
		O	D	O	D	O	D	O	D	O	D	O	D
SS11	1640	7.9	11.7	4.5	5.9	8.1	7.3	10.0	7.1	10.0	8.3	8.0	11.2
SS12	1018	4.3	4.4	5.9	4.4	9.4	11.3	7.1	9.9	12.3	12.4	6.5	12.2
SS13	4455	9.1	6.9	6.6	5.7	9.8	7.3	9.1	8.5	9.3	9.0	9.1	9.6
SS14	1495	5.9	3.5	3.6	3.4	11.6	7.6	7.2	7.3	13.7	13.7	9.4	13.3
SS15	128	4.7	0.8	0,2	2.5	4.5	11.0	3.5	3.9	15.7	17.5	15.1	20.6
SS16	1208	4.2	2.6	3.5	2.4	7.7	6.7	8.1	7.7	14.2	13.3	15.2	14.3
SS17	1500	3.4	3.7	4.0	4.2	8.2	7.0	9.6	10.3	13.7	9.1	14.0	12.8
SS18	3420	2.9	3.3	4.1	3.2	9.4	5.7	10.0	7.7	9.3	14.6	15.0	14.7
SS19	2456	5.6	4.2	5.2	4.9	9.9	6.8	6.9	10.1	11.5	10.0	9.9	15.1
SS20	564	2.3	3.4	3.5	3.1	3.6	6.1	6.3	15.7	13.2	9.5	13.7	19.6
SS21	1060	2.6	1.8	2.5	2.4	9.5	4.6	8.5	11.0	16.0	6.7	13.8	20.6
SS22	2703	3.8	4.1	3.6	4.4	8.9	8.2	7.6	10.0	12.6	10.5	13.4	12.9
X:		4.7	4.2	3.9	3.9	8.4	7.5	7.8	9.1	12.6	11.2	11.9	14.7
SE:		0.6	0.8	0.5	0.4	0.7	0.6	0.6	0.8	0.6	0.9	0.9	1.0
								t = 1.37		t = 1.37		t = 3.18	
								n.s.		n.s.		p < .01	

*O = Placebo
D = DOCA

Experiment 13

Rat	ARs per NPS+PS pair of sessions	Percent of ARs: 1st 15 min NPS	1st 15 min PS	2nd 15 min NPS	2nd 15 min PS	3rd 15 min NPS	3rd 15 min PS
I. Thirty Minute Delay in Home Cage after Prestress							
N33(1)*	355	6.3	8.9	17.0	19.2	20.1	28.5
N34(4)	742	6.7	7.2	17.1	18.4	25.7	24.3
N35(2)	35	3.6	2.9	9.4	15.8	29.5	38.8
N36(4)	1183	6.6	5.0	15.3	22.4	20.2	30.4
N37(2)	257	1.6	0.2	10.9	27.5	30.4	29.6
N38(2)	847	10.6	2.5	18.2	20.8	22.5	25.4
N39(3)	15	16.7	11.7	21.7	21.7	15.0	13.3
N40(4)	6482	6.6	7.1	18.2	22.4	21.0	24.7
N41(1)	1437	6.7	7.0	19.0	20.7	21.6	25.0
N42(3)	405	5.7	5.3	9.4	10.7	26.2	42.7
N43(2)	4299	6.5	8.4	17.7	15.9	26.5	25.0
N44(3)	1045	2.6	4.0	20.0	18.2	27.5	27.7
N45(3)	380	7.3	6.7	17.4	22.0	21.6	25.0
N46(1)	74	26.9	19.5	8.8	7.7	21.2	15.8
N47(4)	1303	1.5	4.7	12.0	17.7	26.4	37.7
N48(1)	17	11.8	19.1	8.8	26.5	10.3	23.5
X:		8.0	7.5	15.1	19.2	22.9	27.3
SE:		1.6	1.3	1.1	1.3	1.3	1.9
				t = 2.85		t = 2.84	
				p < .01		p < .01	
II. Five Minute Delay in Home Cage after Prestress							
N33(2)	148	13.0	14.2	12.9	16.1	14.5	29.2
N34(3)	586	4.5	14.2	7.1	30.2	11.0	32.9
N35(4)	18	8.3	18.1	5.6	8.3	30.6	29.2
N36(3)	1166	8.6	8.7	15.7	20.8	22.7	23.4
N37(1)	760	1.5	1.3	21.9	17.4	32.8	25.2
N38(1)	919	2.0	12.4	7.5	30.4	21.9	25.8
N39(4)	258	5.1	5.8	11.5	17.0	25.9	34.8
N40(2)	7244	8.1	7.9	15.7	20.1	22.8	25.4
N41(3)	1383	5.8	8.6	17.3	16.7	23.7	28.0
N42(1)	522	13.8	13.9	12.7	16.7	23.7	28.0
N43(4)	2223	3.9	4.1	11.5	17.3	28.1	35.0
N44(4)	932	7.6	7.8	20.1	16.6	23.5	24.4
N45(1)	422	10.3	16.4	12.0	11.4	24.5	25.5
N46(3)	110	3.4	3.9	24.5	23.6	14.8	29.8
N47(2)	2602	1.5	3.0	11.5	20.3	27.1	36.6
N48(2)	20	9.0	7.7	19.5	15.4	23.1	25.6
X:		6.7	9.3	14.2	19.1	22.5	28.4
SE:		1.0	1.3	1.4	1.5	1.6	1.1
		t = 2.66		t = 2.44		t = 3.25	
		P < .01		p < .05		p < .005	

Experiment 13 (continued)

III. One Minute Delay in Home Cage after Prestress

N33(4)	235	9.4	17.4	17.0	19.9	11.0	25.4
N34(1)	1082	7.2	7.1	19.6	13.2	29.2	23.7
N35(1)	222	3.0	5.4	11.4	17.4	23.8	38.9
N36(2)	2192	7.4	9.5	16.0	20.3	22.8	24.1
N37(3)	128	6.0	2.3	15.4	14.6	27.3	34.1
N38(4)	529	11.0	16.8	12.2	24.3	18.5	17.1
N39(1)	102	19.7	26.6	10.3	18.7	16.0	8.6
N40(1)	9206	9.1	10.3	19.6	16.5	22.4	22.0
N41(2)	1509	6.8	10.3	16.8	20.8	19.6	25.7
N42(2)	606	8.8	10.9	17.6	17.9	15.8	29.0
N43(3)	3562	4.4	4.9	16.8	10.9	28.3	34.6
N44(2)	1383	4.8	6.5	17.6	17.9	15.8	29.0
N45(4)	711	4.5	18.2	13.6	22.0	14.6	27.1
N46(4)	177	7.3	6.9	7.8	31.6	9.5	37.0
N47(3)	1862	2.8	2.7	14.9	15.1	33.3	31.2
N48(3)	54	3.7	7.4	10.2	20.4	32.4	24.1
X:		7.2	10.2	14.8	18.9	21.8	26.9
SE:		1.0	1.6	0.9	1.2	1.8	1.9
		t = 2.89		t = 2.10		t = 2.08	
		p < .01		p < .05		p < .05	

IV. No Return to Home Cage after Prestress

N33(3)	91	1.7	12.1	35.0	20.1	15.2	16.0
N34(2)	931	3.6	8.9	13.6	25.0	21.0	27.9
N35(3)	7	6.9	13.8	17.2	6.9	27.6	27.6
N36(1)	2212	5.6	8.5	17.8	16.0	28.0	24.0
N37(4)	217	5.5	2.9	14.3	13.0	33.8	30.5
N38(3)	544	17.0	4.9	9.9	29.0	22.7	16.6
N39(2)	67	6.7	23.1	10.4	11.9	35.8	11.9
N40(3)	6400	8.0	8.4	21.4	18.4	23.5	20.4
N41(4)	1064	7.8	11.5	19.9	20.7	22.4	17.6
N42(3)	405	11.0	11.0	16.2	16.3	25.3	20.2
N43(1)	5863	8.7	11.8	17.7	18.4	21.1	22.3
N44(1)	1301	4.7	3.8	13.8	14.3	23.5	39.9
N45(2)	600	11.9	24.3	1.7	26.8	3.7	31.7
N46(2)	455	12.4	8.6	15.1	13.1	41.2	9.7
N47(1)	4820	5.3	8.8	16.4	16.5	26.4	26.6
N48(4)	94	13.6	11.2	19.5	18.4	22.2	15.0
X:		8.2	10.9	16.2	17.8	24.6	22.4
SE:		1.0	1.5	1.7	1.4	2.1	2.0
		t = 1.57					
		n.s.					

*The number (1,2,3, or 4) in parentheses after each subject indicates the position in the sequence of test blocks at which the subject was tested in the delay condition indicated by the table.

Experiment 14

Rat	ARs per NPS+PSO+PS1 triplet of sessions	Percent of ARs								
		NPS	PSO	PS1	NPS	PSO	PS1	NPS	PSO	PS1
Normal rats (sham adrex)										
N61	7867	8.0	5.6	5.7	14.4	11.7	10.7	14.0	14.5	15.4
N62	3567	6.9	6.9	7.1	11.9	9.9	11.9	13.9	13.4	18.0
N63	3479	4.5	5.9	5.3	11.7	10.8	13.2	13.7	18.4	16.4
N64	799	1.3	2.3	3.7	4.4	9.6	13.5	15.7	22.9	26.5
N65	284	7.6	11.6	25.1	5.4	4.1	20.0	3.9	6.6	15.7
N66	516	7.1	6.5	9.9	10.7	11.4	12.4	14.7	16.1	11.1
N67	1737	1.9	5.5	3.8	8.3	11.4	9.8	16.3	19.4	23.5
N68	6640	5.8	7.2	6.5	10.6	10.4	10.2	17.8	14.3	17.1
X:	3-97	5.4	6.4	8.4	9.3	9.9	12.7	13.8	15.7	18.0
SE:	1011	0.9	0.9	2.5	1.3	0.9	1.2	1.5	1.7	1.7
			n.s.			n.s			t = 2.20 p < .05	
Adrex rats										
Ax61	1408	9.3	9.5	6.8	10.0	8.8	9.6	15.0	13.7	17.3
Ax62	6394	3.3	5.9	8.1	10.4	13.1	10.0	16.0	15.8	17.0
Ax63	1745	6.0	6.9	6.9	12.1	10.7	11.5	16.8	14.9	13.9
Ax64	551	2.4	6.7	8.3	7.4	9.7	14.2	8.1	15.9	26.8
Ax65	490	4.2	3.7	5.7	5.1	7.9	12.6	8.4	15.8	26.8
Ax66	670	1.0	4.1	0.3	6.7	11.6	9.1	16.9	29.9	20.3
Ax67	4446	3.5	6.6	6.7	10.3	12.7	13.1	13.8	14.9	18.4
Ax68	1259	6.5	6.5	11.3	13.4	12.3	13.6	8.3	11.6	16.7
X:	2120	4.5	6.2	6.8	9.4	10.9	11.7	12.9	16.6	20.8
SE:	759	0.9	0.6	1.1	1.0	0.7	0.7	1.4	2.0	2.5
		t - 2.73 p .05				n.s.		t = 1.95 p .05		
		t = 2.76 p < .05			t = 1.98 p < .05			t = 2.18 p < .05		

Experiment 15

Rat	ARs per NPS+PS1+PS30 triplet of sessions	Percent of ARs: 1st 15 min NPS	1st 15 min PS1	1st 15 min PS30	2nd 15 min NPS	2nd 15 min PS1	2nd 15 min PS30	3rd 15 min NPS	3rd 15 min PS1	3rd 15 min PS30
Normal rats (sham adrex)										
N71	325	4.2	5.9	4.8	11.9	16.4	8.5	10.5	24.2	13.6
N72	2589	7.5	5.7	5.5	11.0	8.7	15.2	14.2	15.5	16.7
N73	2073	4.0	5.0	5.3	10.6	11.1	16.3	14.7	12.0	21.0
N74	694	8.4	7.6	6.6	8.6	10.2	8.6	15.4	16.7	17.7
N75	83	5.8	8.6	7.0	9.6	14.0	13.4	14.0	9.8	17.6
N76	3057	7.5	9.3	8.7	11.0	9.4	12.3	14.1	13.3	14.4
N77	2533	9.1	8.4	6.9	10.8	13.7	11.7	13.3	13.7	12.6
N78	1596	6.0	16.2	8.8	8.6	8.6	13.4	5.5	17.4	15.5
N79	2553	7.1	10.5	8.4	10.1	12.5	12.1	12.8	13.6	12.9
X:		6.6	8.6	6.9	10.2	11.6	12.4	12.7	15.1	15.8
SE:	367	0.6	1.1	0.5	0.4	0.9	0.9	1.0	1.4	0.9
		t = 1.66 n.s.			t = 1.66 n.s.			t = 1.68 n.s.		
			t = 2.24 p < .05			t = 2.27 p < .05			t = 2.73 p < .05	
Adrex rats										
Ax71	1226	3.2	3.1	3.3	7.4	8.8	8.8	21.3	23.7	20.4
Ax72	4021	6.3	6.6	5.2	13.1	13.0	11.9	14.6	15.6	13.7
Ax73	825	5.3	5.7	7.8	7.5	10.1	13.6	13.8	16.5	19.8
Ax74	571	4.4	4.9	3.0	10.3	9.5	10.9	11.2	25.9	20.0
Ax75	578	7.8	13.7	5.7	10.6	10.2	10.7	12.8	14.5	14.0
Ax76	2982	9.6	9.5	6.6	13.1	10.3	8.9	14.7	14.8	12.5
Ax77	1053	6.4	10.9	6.6	11.9	12.4	8.9	12.4	17.2	13.3
Ax78	733	5.3	8.7	5.6	12.7	16.5	8.0	10.5	16.2	16.4
Ax79	2257	7.5	9.7	7.8	10.1	11.7	9.0	15.6	15.6	12.8
X:	1583	6.2	8.1	5.7	10.7	11.4	10.1	14.1	17.8	15.9
SE:	410	0.6	1.1	0.6	0.7	0.8	0.6	1.1	1.4	1.1
		t = 2.45 p < .05			n.s.			t = 2.49 p < .05		
			t = 2.49 p < .05						t = 2.15 p < .05	

End