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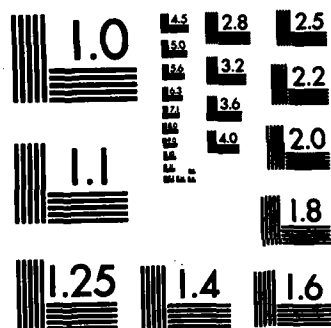
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BEHAVIORAL AND PHYSIOLOGICAL EFFECTS OF PSYCHOLOGICAL STRESS IN RATS

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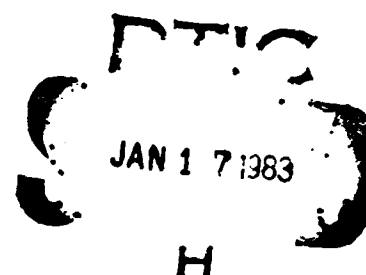
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Aerospace Medical Division (AFSC)

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NOTICES

This final report was submitted by Systems Research Laboratories, Inc., 2800 Indian Ripple Road, Dayton, Ohio 45440-3696, under contract F33615-80-C-0603, job order 7757-05-43, with the USAF School of Aerospace Medicine, Aerospace Medical Division, AFSC, Brooks Air Force Base, Texas. Captain Thomas E. Dayton (USAFSAM/RZW) was the Laboratory Project Officer.

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The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources - National Research Council.

The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service, where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.



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BEHAVIORAL AND PHYSIOLOGICAL EFFECTS OF PSYCHOLOGICAL STRESS IN RATS

INTRODUCTION

In a military emergency, presumably a high degree of stress will come from several sources: (a) The psychological reality of the emergency condition (attack, threat of attack, etc.); (b) the physical and mental demands on military personnel responding to the emergency; and (c) the effects of direct physical insult (e.g., exposure to ionizing radiation, chemical agents, or antidotes). This study evaluates an animal's ability to learn a new task after varying degrees of psychological stress, and determines the extent of physiological damage which the stress produced.

The body's reaction to psychological and physiological stressors is similar. Every stressing agent creates nonspecific biological effects characteristic of stressors in general, as well as some effects specific to the particular stressing agent. Cannon (10) and Selye (34) have described the way in which exposure of an animal to noxious stimuli results in activation of the neuroendocrine system. Cannon (10) emphasized the activation of the sympathetic nervous system and the release of epinephrine from the adrenal medulla in facilitating an emergency behavioral reaction. Selye (34) pointed to the release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary (with a subsequent increase in circulating adrenocortical steroids) as reflecting alterations, in physiological homeostasis, that underlie the ability of the organism to adapt to the environment. Normal pituitary-adrenal feedback mechanisms function to maintain the organism in a balanced metabolic state. Stress overrides the normal feedback system to mobilize resources for emergency functioning. Long-continued exposure to stress creates an unbalanced metabolic state, resulting in shortened lifespan and various diseases of adaptation (34).

Although the fact is less emphasized, the reticuloendothelial system (RES) is also involved during stress in interactions with the autonomic nervous system and the pituitary-adrenal system. The RES is the blood immune system, which has been shown to respond both specifically (to isolate particular pathogens) and nonspecifically as a defense against any stressor (36, 41). An intact pituitary-adrenal system is necessary to maintain the capacity of the RES to respond to foreign substances (14). Some of the steroids secreted by the adrenal cortex in response to ACTH stimulate RES functions, while others depress the RES. Nicol et al. (26) found that estrogen was the strongest RES stimulant, whereas corticosteroids were strong depressants. Testosterone and progesterone had little effect. For some time, a relationship has been recognized between avoidance conditioning and the pituitary-adrenal hormonal response to stress (12, 13).

Recently attempts have been made to show that behaviorally induced escape learning decrements are associated with the neuroendocrine effects of stress. Behavioral deficits induced by inescapable shock are due to a

reduction in brain norepinephrine and dopamine and to an increase in acetylcholine levels (1, 3, 38). Pharmacological treatments that deplete central catecholamines mimic the behavioral effects of inescapable shock, whereas treatments that increase catecholamine activity eliminate such effects (2, 4, 17). Death, resulting from the stress of an uncontrollable environmental contingency, has been shown to result from sympathetic over-stimulation of the heart and adrenergic depletion (32).

In rodents, immobilization decreases adrenal catecholamine content, while elevating plasma epinephrine and norepinephrine levels (20, 21). Immobilization also reduces immune responsiveness (11). Immobilization produces ulcers in rodents, a typical response to psychological stress (15). More ulcers are produced by increasing the restriction time or reducing the degree of movement (7, 8, 9). Restraint is synergistic in the production of ulcers with such other stressors as food deprivation, cold temperatures, and electric shock (18, 35, 37).

Studies also attest to the psychological nature of immobilization stress. The availability of "prepared responses" (opportunity to struggle or gnaw on wood) decreases the number of ulcers (18); and making restraint sessions predictable reduces the magnitude and duration of plasma changes (31). From a logical viewpoint, immobilization would appear to meet the criterion that a stressor be "biologically significant" (32). In the rodent's natural environment, severe movement restriction probably signifies entrapment or capture by a predator; and an unsuccessful struggle may be the last response preceding death.

Sex differences have been found in physiological responses to stress. The appropriate stressor should thus produce differential behavioral effects between males and females. Specifically, females should be more stress-resistant, because they have a greater amount of estrogen which stimulates immune responses (27). Park and Scarborough (30) have shown that conditioned emotional responding in a group of adrenalectomized and gonadectomized animals could be restored to normal by giving them a form of artificial estrogen. Significant differences in simple two-way avoidance conditioning, in which males show lower levels of avoidance behavior, appear after 90 days of age (5). These gender differences may be exacerbated by stress.

Numerous reports have been written on the avoidance conditioning deficits following manipulation of physiological stress parameters (13). Similar deficits have been reported in dogs when uncontrollable shock was used as a psychological stressor. Deficits in two-way avoidance acquisition have not been shown in rats, although escape deficits can be produced by complicating the reinforcement contingencies (22, 23, 24, 27, 33). Despite much theoretical speculation, we still do not know why rats have not shown learning decrements in the typical avoidance paradigms after uncontrollable shocks. Even though previous studies have not shown deficits in avoidance acquisition in rodents, immobilization stress induces profound physiological changes; and we predicted that it would produce analogous learning deficits.

Using immobilization as an environmental stressor for the rat, this study looked for changes in activity level and the ability to acquire a simple two-way avoidance response. The parameters of this effect were

investigated in both male and female rats by varying the duration of the immobilization (degree of stress). After the behavioral testing, the animals were sacrificed to determine if changes had occurred in adrenal weights or in the incidence of stomach ulcers.

METHODS

Presented in this report section is information on how the subjects, apparatus, and procedures were used in the research.

Subjects

The subjects were 54 male and 54 female Sprague-Dawley rats, obtained at 72 days of age from Harlan Industries, Indianapolis, Indiana. They were given *ad libitum* access to food and water, and were maintained on a 12-hr-on and 12-hr-off light-dark cycle for 4 weeks prior to experimentation.

Apparatus

Activity testing was performed in two standard open-field mazes, 3 ft (91.4 cm) square with a wall 1-ft (30.5 cm) in height. Nine 1-ft squares were marked on the maze floor. The maze was constructed of plywood, painted with glossy white enamel for ease of cleaning between trials. The mazes were located in the center of a large room, approximately 2.6 m beneath a bank of 40-W fluorescent bulbs. Observers were seated on stools at opposite corners of each maze. No obvious shadows were cast on the maze floor by either of the observers or by the maze walls.

Avoidance testing was performed in three shuttle-boxes modeled after Lafayette Instrument Company's "Modular Testing Unit 8500." The two compartments of each shuttle-box had a floor of stainless steel bars that could be electrified independently. The compartments were connected by an opening, and the weight of a rat on the floor grid closed a switch to indicate the subject's location. Shock was delivered to each grid by a Coulbourn Model E13-16 Shock Distributor. Small, dim, incandescent lamps were on the end walls of each chamber; and two brighter incandescent lamps were in the ceiling of each compartment above Plexiglas diffusers. The top half of the front wall of the chamber was made of half-silvered ("one-way") glass, to allow the experimenters to observe the subject. The shuttle-boxes were interfaced to a laboratory computer (NOVA 800) through a control panel (MANX, G. C. Controls, Inc.). The schedule and the duration of stimuli delivered to the rat were programmed, and the rat's responses were also recorded by the computer. A general description of the automated testing and data acquisition system used in these experiments has been published (6). The specific details of hardware and software for these experiments are presented in Appendixes A to D.

Procedure

Every animal was handled and weighed, once each day, for 5 days. At approximately 100 days of age, 9 male and 9 female subjects were randomly assigned to each of the experimental conditions. Serving as controls were 18 male and 18 female subjects. The groups were defined by the number of hours of restraint stress received: 0, 2, 8, 14, or 18 hr. Half of the control subjects (0 hr restraint) were tested at the beginning of the sequence; and the other half, at the end. The remaining groups were tested in the following sequence: 18, 2, 14, and 8 hr.

Before testing, all groups were deprived of food for 24 hr, and water for 18 hr. Each animal was subjected to restraint stress by being wrapped in screen wire for the prescribed number of hours prior to testing. This wrapping was accomplished by placing the rat near the edge of a piece of hardware cloth, and then rolling the cloth into a tube shape which surrounded the rat. Sheets of 1/16 in. (1.5875 mm) aluminum hardware cloth, approximately 17.7 in. (45 cm) long, were used to restrain the subjects. The ends of the screen wire tube were twisted and secured with 20-ga galvanized wire. Care was taken to wrap the screen wire tight enough that the subject would be immobilized, but not so tight as to compress or twist the animal into an abnormal position. Each wrapped animal was placed in a plastic tray in a normal upright position. Experimental subjects were always restrained in a separate room and never returned to the colony because of the potential for stress communication through pheromone production. By the same logic, experimental and control animals were never tested on the same day. The apparatus was water-sponged after each test. Additionally, the apparatus was washed with alcohol and allowed to dry overnight, to minimize odor transfer between groups.

Prior to testing in the activity maze, each animal was placed in a transport cage for 10 min with access to water. Then the animal was placed in the center square of the open-field maze, and the following data were recorded independently by two observers for 5 min: (a) latency to leave the center square; (b) total number of lines crossed (ambulations); (c) number of center square crossings; (d) grooming episodes; (e) rearing episodes; and (f) number of fecal boli excreted. Interobserver reliability was high, with differences averaging less than 2 counts in 50 observations; and, therefore, the responses recorded by each pair of observers were averaged for subsequent analysis.

After activity measurement, each animal was taken (via a transport cage) to an adjacent room for escape and avoidance testing. Escape and avoidance testing occurred in a darkened room, and began with the animal in the shuttle chamber for a 5-min adaptation period prior to testing.

The small end-wall lamps were "ON" during the adaptation period, thus permitting the experimenter to observe the rat. The overhead lamps on each side of the chamber were programmed to be the primary conditioned stimulus, since they were more salient than the end lamps. The program consisted of a 5-sec conditioned stimulus (CS) period, during which both the overhead and end-wall lights flashed at a 2-Hz rate in the side of the chamber where the rat was located. If the rat did not move to the other compartment of the shuttle-box, an unconditioned stimulus (US)--consisting of a (30-ms duration) pulsed 0.2 mA shock--was administered at a 2-Hz rate (synchronous with the

light flashes) until the animal moved to the other compartment, or until 15 sec had elapsed. The CS (and the US, if present) terminated when the animal moved to the other compartment. If a rat stood in the doorway with its feet on both grids, shock was briefly delivered to both grids in sequence, and the CS was delayed 10 sec. CS-CS intervals were randomly selected from a range of 25 - 55 sec, with a mean of 40 sec. Each animal received 60 trials. Latency to respond from CS onset was recorded for each trial. The trials in each session were also categorized and counted as either: (a) failure to respond (no response for the 20-sec CS-US duration); or (b) escape (response during the US); or (c) avoidance (response during the CS). The number of trials required to reach an arbitrarily set avoidance criterion of 8 avoidances in 10 trials also was recorded.

Within 1 hr after avoidance testing, each animal was sacrificed (Halothane overdose). The stomach was removed, opened, and examined for ulcers, which were specified by the number and approximate length of the lesions.

The SAM Comparative Pathology Branch (VSP) completely necropsied several animals from each group to assess the general health of the animals. If sick animals were found, the results from the entire group were discarded and another group was used in their place.

RESULTS

In this report section, information is presented concerning activity, avoidance, adrenal weights and gastric lesions, and the relationships between lesions and behavior.

Activity

Each of the six measures from the activity maze (latency to leave center square, ambulations, rearing, center square crossings, grooming, and fecal boli) was entered into a separate 2 X 5 factorial analysis of variance. Shown in Table 1 are the results of these analyses in terms of probabilities associated with the null hypotheses. (The effects and interactions are presented graphically in Figs. 1 - 5.)

Dunnett's procedure (41) was used to compare each restraint group (within each sex) to its unrestrained control group. The results of these multiple comparisons are indicated in Figures 1 - 5.

Examination of the data provides a general confirmation of the main hypothesis. Restraint significantly changed the activity of the animals in all but one of the categories, with the greater changes generally occurring with longer restraint times. Since all animals had been food-deprived for 18 hr before the observation took place, fecal boli were so infrequent that this

measure proved to be unreliable (Table 1). The majority of these changes were in the direction of reduced activity for the restrained animals. In general, animals restrained for 2 hr did not show activity levels different from those of controls. Sex differences were noted, with the females tending to show higher activity levels and smaller response changes after restraint. The significant sex by restraint interaction generally arose from the fact that changes in activity of males were largest at 8 or 14 hr of restraint, while the changes for females were generally maximal after 18 hr of restraint.

TABLE 1. PROBABILITY VALUES ASSOCIATED WITH STATISTICAL NULL HYPOTHESES TESTED BY SEPARATE 2 X 5 FACTORIAL ANALYSIS OF VARIANCE FOR EACH BEHAVIORAL MEASURE

Behavioral measure	Experimental variable		
	Restraint duration	Sex	Sex X restraint
Latency to leave center square	.005	.002	.027 (Fig. 1)
Ambulation	< .0001	< .0001	.004 (Fig. 2)
Rearing	< .0001	.0005	.105 (Fig. 3)
Center square crossings	.020	.0001	.003 (Fig. 4)
Grooming	.0001	.705	.204 (Fig. 5)
Fecal boli	.457	.055	.457

Shown in Figure 1 is latency to leave the center square (CSQ) for the two sexes as a function of duration of restraint. The significant sex X restraint interaction (Table 1) arises mainly from the fact that males are much slower to leave the center square after 8 or 14 hr restraint. Females, on the other hand, do not show increased latencies with increased restraint duration. The significant restraint main effect is thus primarily due to the performance of the males.

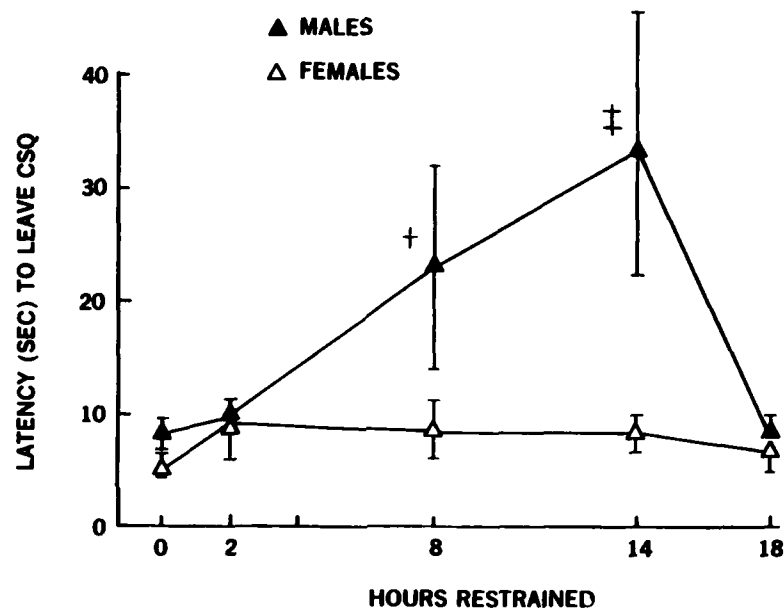


Figure 1. The mean latency (± 1 S.D.) in the activity maze during a 5-min period for male and female rats after 0, 2, 8, 14 and 18 hr of restraint.

Dunnett's procedure was used, for each sex, to test for differences between the unrestrained control group and the various restraint groups. Differences significant: at $\alpha = .05$ are indicated by daggers ($\dagger$); at $\alpha = .01$, by double daggers ($\ddagger$). (CSQ = center square)

Presented in Figure 2 are the ambulation data for the two sexes after varying durations of restraint stress. Females were significantly more active than males. In females, the restraint--which had no effect for the 2-hr duration--reduced activity by increasing amounts as restraint duration increased. For males, also, the restraint had no effect at the 2-hr duration. A large reduction in the ambulatory activity of males occurred after 8 hr of restraint, but longer durations did not appear to increase this effect.

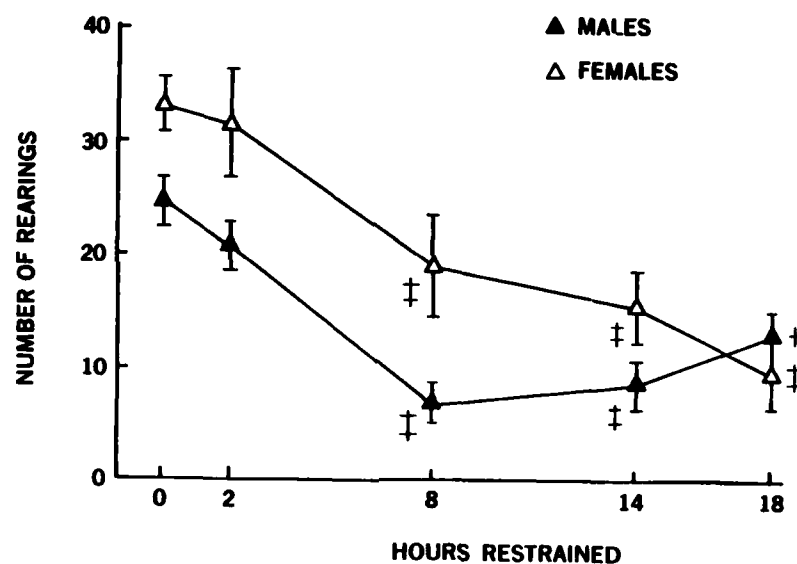


Figure 2. The mean number of lines crossed (± 1 S.D.) in the activity maze during a 5-min period for male and female rats after 0, 2, 8, 14, and 18 hr of restraint.

Dunnett's procedure was used, for each sex, to test for differences between the unrestrained control group and the various restraint groups. Differences significant at $\alpha = .05$ are indicated by daggers (†); at $\alpha = .01$, by double daggers (‡).

Shown in Figure 3 is a similar pattern in another exploratory behavior, rearing. Again, females were more active than males. All except 2 hr of restraint significantly reduced rearing, with the maximal effect occurring at shorter restraint durations for males.

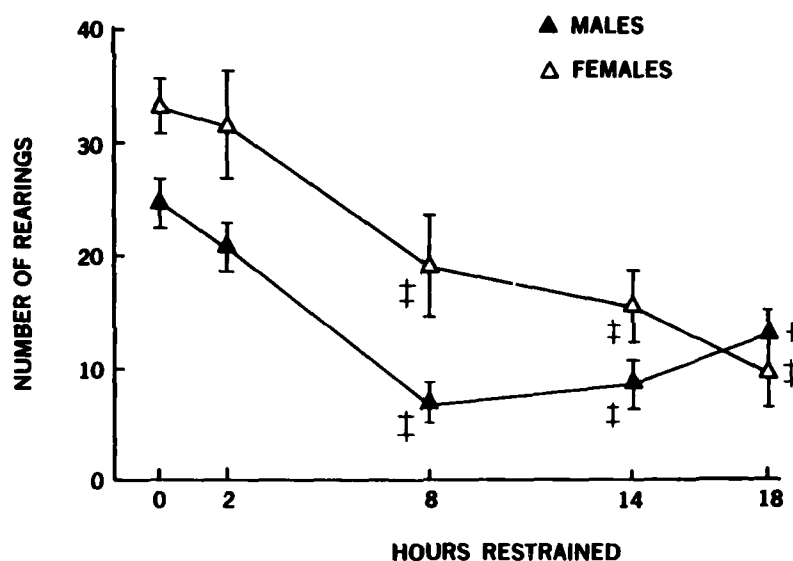


Figure 3. The mean number of rearings (± 1 S.D.) in the activity maze during a 5-min period for male and female rats after 0, 2, 8, 14, and 18 hr of restraint.

Dunnett's procedure was used, for each sex, to test for differences between the unrestrained control group and the various restraint groups. Differences significant: at $\alpha = .05$ are indicated by daggers (+); at $\alpha = .01$, by double daggers (‡).

Shown in Figure 4 is the mean number of times the rats crossed the center square of the activity maze. Overall significant sex differences, effects of restraint duration, and a sex X restraint interaction were observed (Table 1). The males showed a maximal reduction in activity after 8 hr of restraint, while all except the control females exhibited a higher average level of activity than males and increasing reductions in activity with increasing restraint duration.

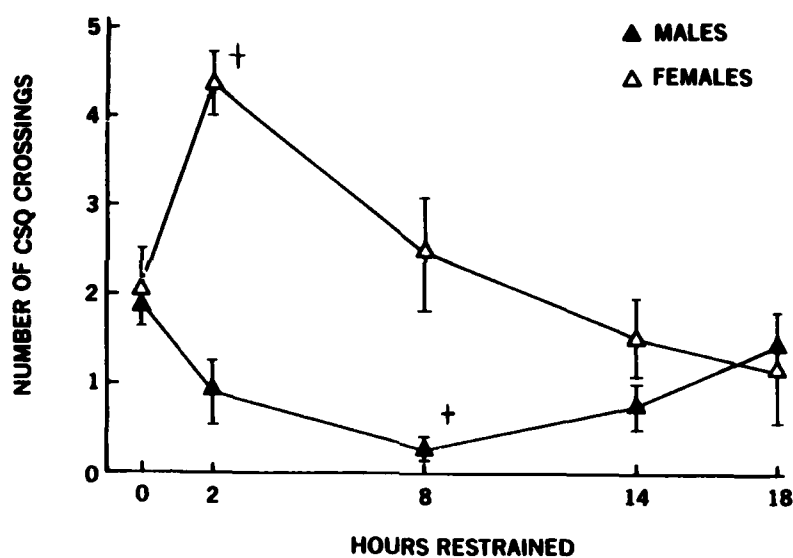


Figure 4. The mean number of center square crossings (± 1 S.D.) during 5 min in the activity maze for male and female rats as a function of restraint stress duration.

Dunnett's procedure was used, for each sex, to test for differences between the unrestrained control groups and the various restraint groups. Differences significant: at $\alpha = .05$ are indicated by daggers (+); at $\alpha = .01$, by double daggers (*). (CSQ = center square)

The grooming data for both sexes as a function of restraint duration is shown in Figure 5. The effect of restraint duration was significant (Table 1), while no sex difference or sex by restraint interaction was found. The major change in grooming associated with restraint is the increase noted at 18 hr.

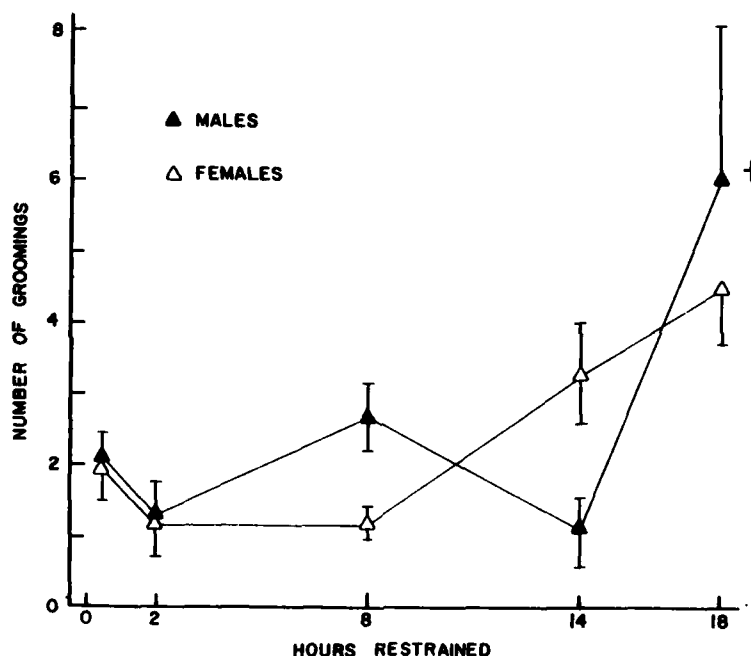


Figure 5. The mean number of grooming episodes (± 1 S.D.) during 5 min in activity maze as a function of restraint stress duration.

Dunnett's procedure was used, for each sex, to test for differences between the unrestrained control groups and the various restraint groups. Differences significant: at $\alpha = .05$ are indicated by daggers (+); at $\alpha = .01$, by double daggers (#).

In summary, the data from the activity maze present a fairly consistent pattern. Females were generally more active than males, and showed larger reductions in activity with increased restraint duration. The performance of males and females was the same after 18 hr of restraint; but the males tended to reach a maximum at 8 or 14 hr, and some improvement in responsiveness at 18 hr. This preliminary finding suggests that females were more resistant to acute stress, whereas males and females could adapt equally well to chronic stress. Further data should be gathered before any definitive statements can be made.

Avoidance. The computer automatically recorded shuttle-box latencies by taking the difference between the light onset (CS) and the rat's response of moving to the opposite compartment of the shuttle-box, where his weight on the grid closed a microswitch. (This latency should be distinguished from the latency to leave the center square of the activity maze, noted in the previous section.) Latency to respond after the CS was recorded for each subject, and was averaged across 6 blocks of 10 trials each. Analysis was by means of a 2 X 5 X 6 factorial repeated measures analysis of variance (main effects of sex, restraint, and blocks of trials) (40).

The learning curves for each group of animals are shown in Figure 6. Latencies for all groups decreased over blocks of 10 trials (repeated measures effect at $p < .0001$). However, the restrained animals had consistently longer response latencies than the control animals ($p < .0001$). Learning rate was, in general, slower with the greater number of hours of restraint; but a maximum performance decrement seemed to occur for animals wrapped for 14 hr (Fig. 7). A 2 X 2 X 6 (control vs. one level of restraint X sex X blocks) repeated measures analysis of variance showed a significant difference in the learning curves (i.e., blocks by groups interaction, $p = .0001$). In all experimental groups, except the group restrained for 2 hr, subjects were initially slower to learn than controls ($p < .001$); by the third block of trials, however, the learning rate was similar. The performance of all groups approached an asymptote, but the performance of the restrained animals was always depressed relative to the control animals ($p < .005$ to $p < .0001$).

Although, in general, the females had shorter mean latencies than males, the learning curves were similar for the two sexes. In Figure 8, this fact is illustrated for the 18-hr restraint vs. control groups. No treatment by sex interactions occurred, as is evidenced by the parallel performance of the males and females. The mean latency of control females was less than 5 sec (avoidance) for the last three blocks of trials (Fig. 8). Although the control males were more likely to wait for the onset of shock to respond (escape, their response latencies approached those of the females.

These data were further separated into three mutually exclusive response categories for additional analysis. Each response was categorized as either: (a) failure to respond (latency greater than 20 sec); (b) escape (latency of greater than 5 sec but less than 20 sec); or (c) avoidance (latency of less than 5 sec). Criterion was defined as 8 avoidances in 10 trials. An analysis of variance for all 60 trials was performed for each of these measures.

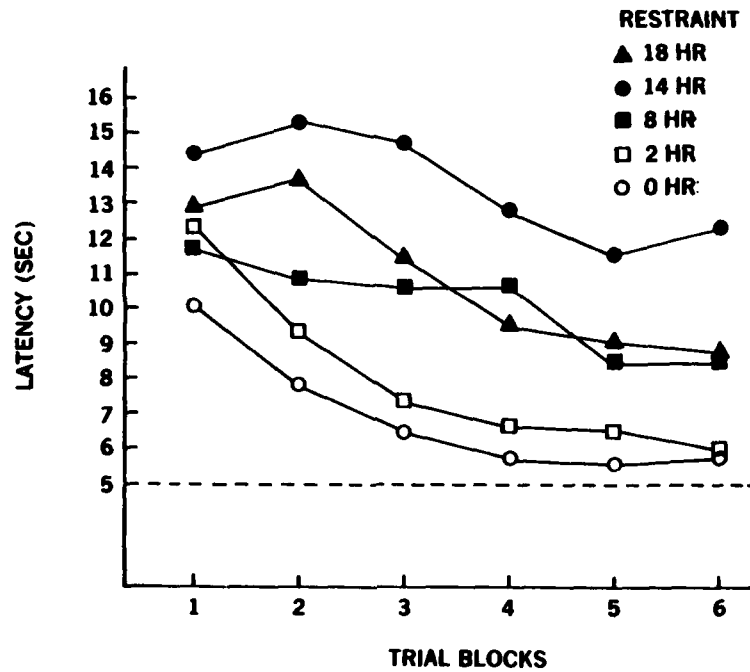


Figure 6. The mean avoidance latencies across blocks of 10 trials for rats subjected to 0, 2, 8, 14, and 18 hr of restraint stress.

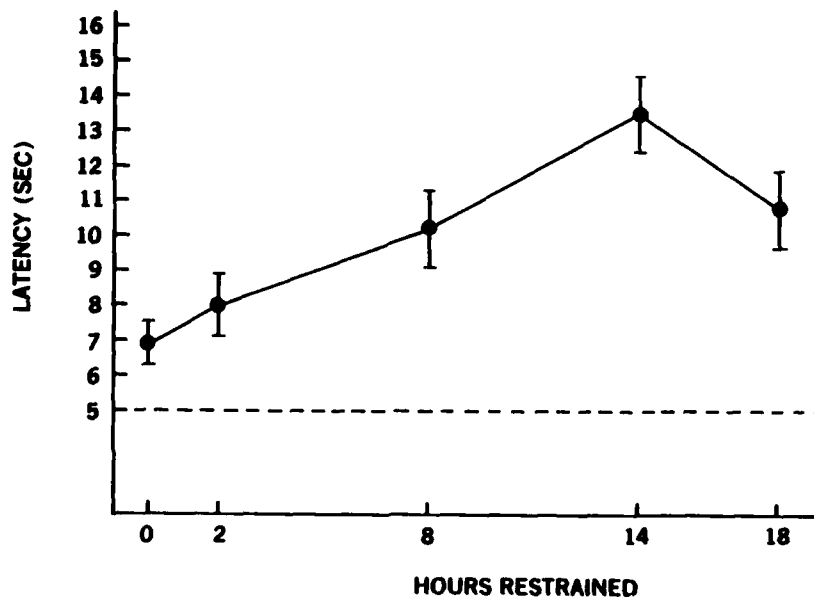


Figure 7. The mean avoidance latency (± 1 S.D.) during 60 shuttle-box trials as a function of restraint stress duration.

Restrained animals showed more failures to respond ($p < .0001$) and fewer avoidances ($p < .0001$) than did the controls. Although the learning curves of restrained and control groups (Figs. 6 and 8) are seemingly similar, grouping into mutually exclusive categories showed that the animals within these groups were making qualitatively different responses (Fig. 9). Restrained animals in the 8-, 14-, and 18-hr treatment groups were more than five times more likely than control animals not to respond on a trial. If the restrained animal did respond, that response was less than half as likely to be an avoidance.

Shown in Figure 10 is the distribution of response times with increasing restraint duration. We noted a decrease in the proportion of escape responses to failures to respond as restraint duration increased (Fig. 10), and an inverse relationship between avoidance responses and failures to respond (Fig. 11). No significant differences occurred in numbers of escape responses as a function of experimental treatment. Because large systematic increases in failures to respond were concomitant with decreases in avoidance, the overall number of escapes had to remain approximately the same. This finding was not merely an artifact of the classification system, but evidence that animals in all groups were capable of responding and were exposed to the escape contingency.

Control animals were more likely to reach a criterion of 8 avoidances in 10 trials than were the restrained animals ($p < .01$). Very few animals reached criterion, and the number was easily reduced by any level of stress. None of the males or females reached criterion in the 14- and 18-hr conditions. The control females reached criterion in fewer trials than the males. However, the females in the 2- and 8-hr groups took slightly more trials than the males to reach the same criterion (interaction $p < .01$).

Although we previously noted no overall significant differences in response latencies between sexes, an analysis by response category showed that females were significantly more likely to avoid than were the males ($p < .05$) (Fig. 12). That response difference was most pronounced in the control animals.

In conclusion, restraint stress produced decrements in two-way shuttle-box avoidance conditioning. When compared to the controls, the stressed rats had longer escape and avoidance latencies, failed to respond more often, avoided shock less frequently, and learned more slowly.

Adrenal Weights and Gastric Lesions

After behavioral testing, the animals were sacrificed, their adrenal glands removed for weighing, and their stomachs examined for lesions. The adrenal glands were temporarily placed in cold saline. Residual fat was carefully removed from the glands, and they were individually weighed within 2 - 4 hr after their removal. An analysis of variance on these weights showed no significant effects.

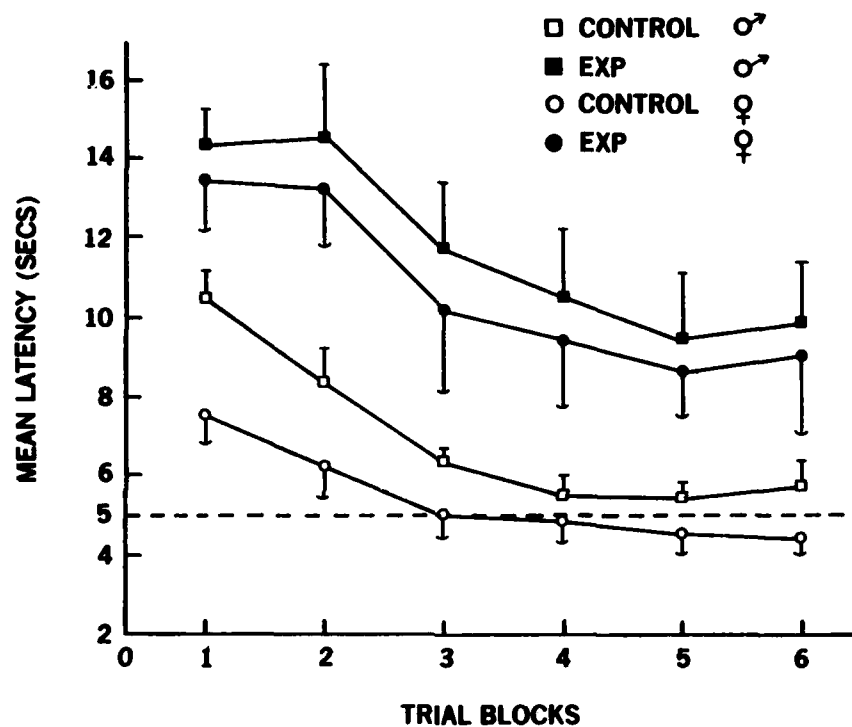


Figure 8. The mean avoidance latency (± 1 S.D.) for male and female control and male and female experimental rats, restrained for 18 hr during blocks of 10 trials in the shuttle-box.

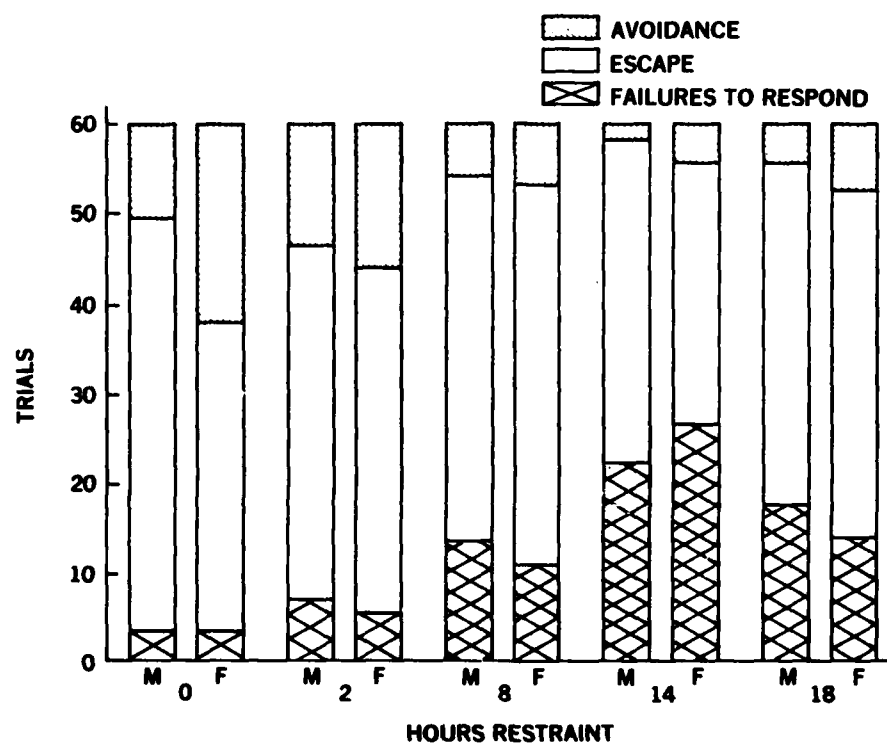


Figure 9. The number of avoidances, escapes, and failures to respond during 60 trials by male and female rats as a function of restraint stress duration.

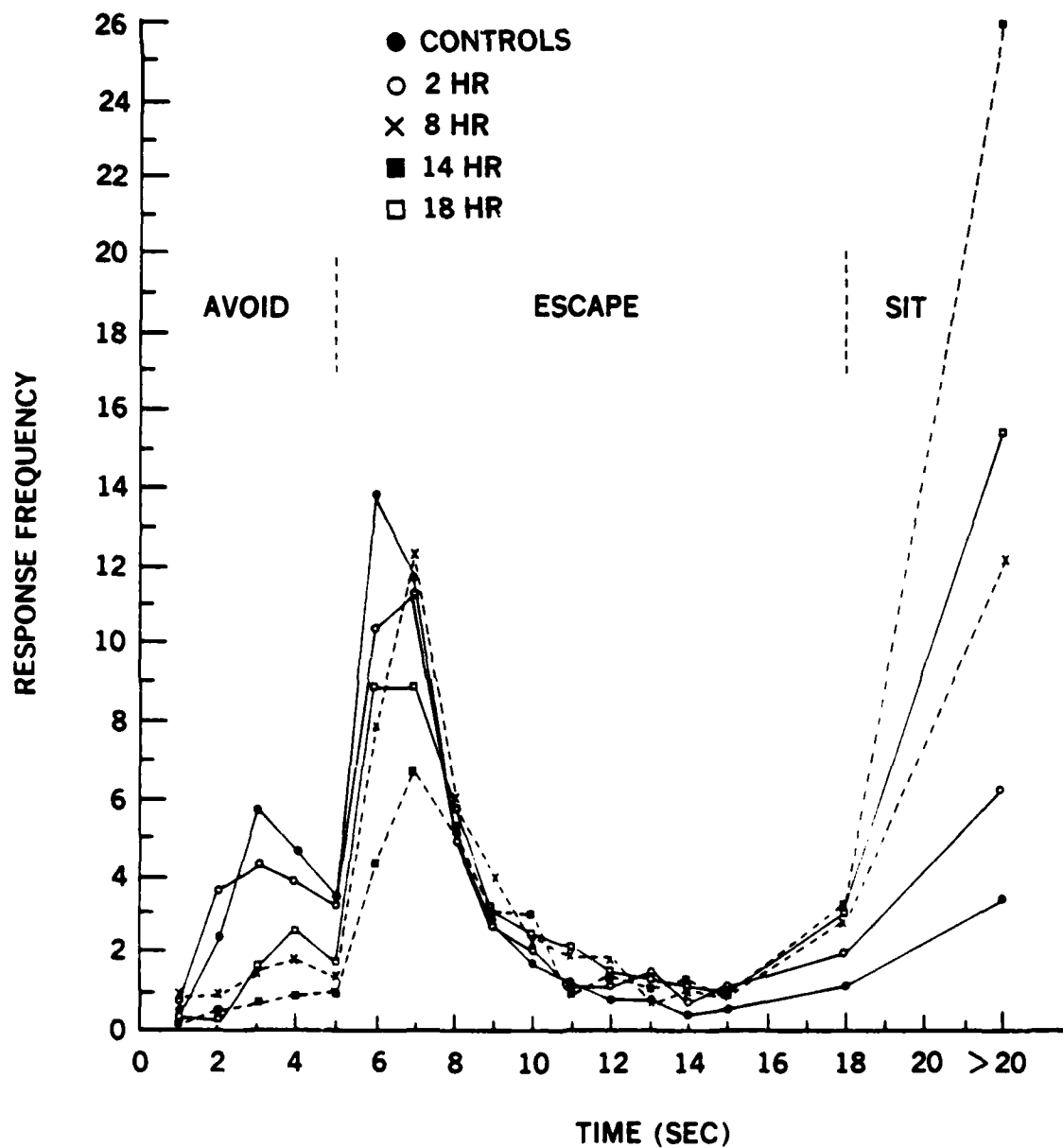


Figure 10. The mean number of responses in each second after CS onset for each group of animals (controls, 2, 8, 14, and 18 hr restrained).

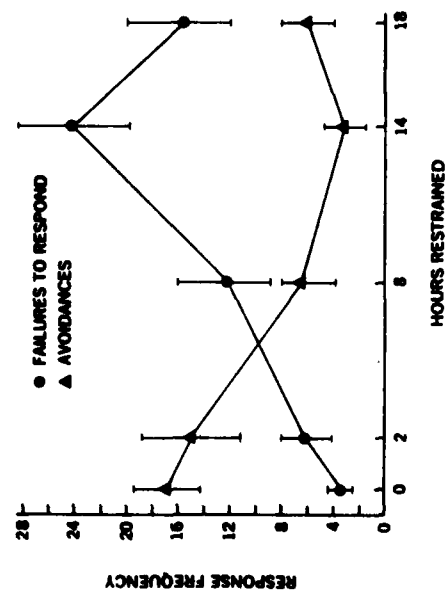


Figure 11. The mean number (± 1 S.D.) of failures to respond and avoidances as a function of restraint stress duration.

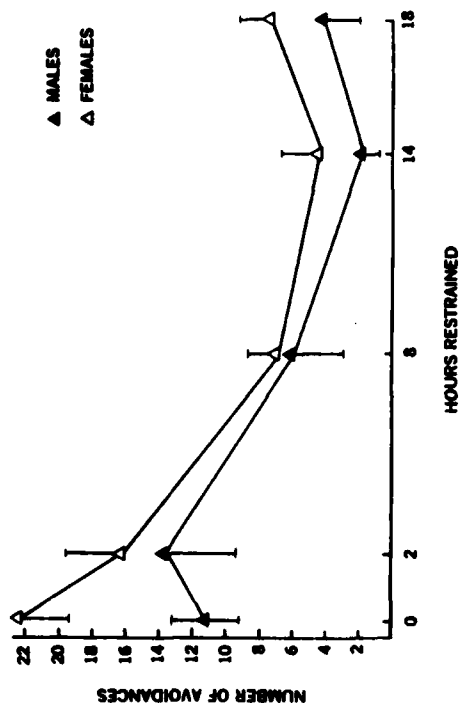


Figure 12. The mean number (± 1 S.D.) of avoidances shown by male and female rats after 0, 2, 8, 14, and 18 hr of restraint stress.

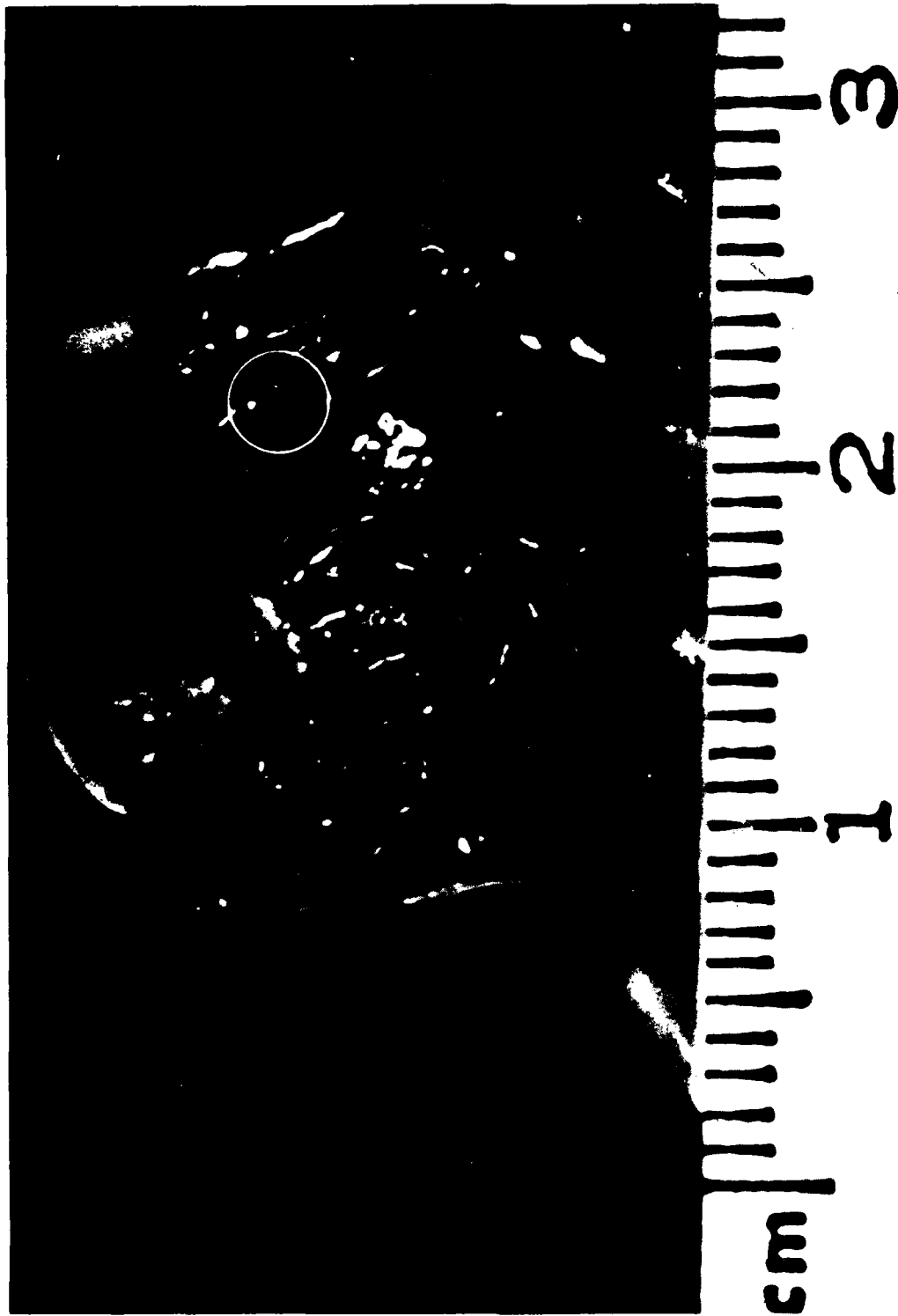


Figure 13. A rat's stomach, with the general location and appearance of a lesion shown after 14 hr of restraint stress.

A stomach lesion typically consisted of an erosion of the first mucosal layer of the ruminary area of the stomach. Histological examination of several lesions showed breakage of small capillaries and accompanying hematomata. The size of the lesions varied from pinpoint to approximately 2 mm, but they rarely penetrated the stomach wall. The typical lesion is shown, in Fig. 13, as a darkened area between the ruminal folds. An exact size measurement proved difficult, so only the number of observed lesions was recorded.

An analysis of variance showed a significant main effect for the restraint treatment ($p < .0001$). The number of lesions increased with the number of hours of restraint stress, but the slight differences between the number of lesions at 14 and 18 hr of restraint may indicate an asymptotic level of effect (Fig. 14). Longer restraint times were not tested; for one animal died after 14 hr of restraint, and pilot data indicated that as many as half of the animals might die during 24 hr of restraint. Approximately 45% of the 14- and 18-hr restrained animals had gastric lesions, whereas only 5% of the control and the 2-hr restrained animals showed evidence of gastric erosion.

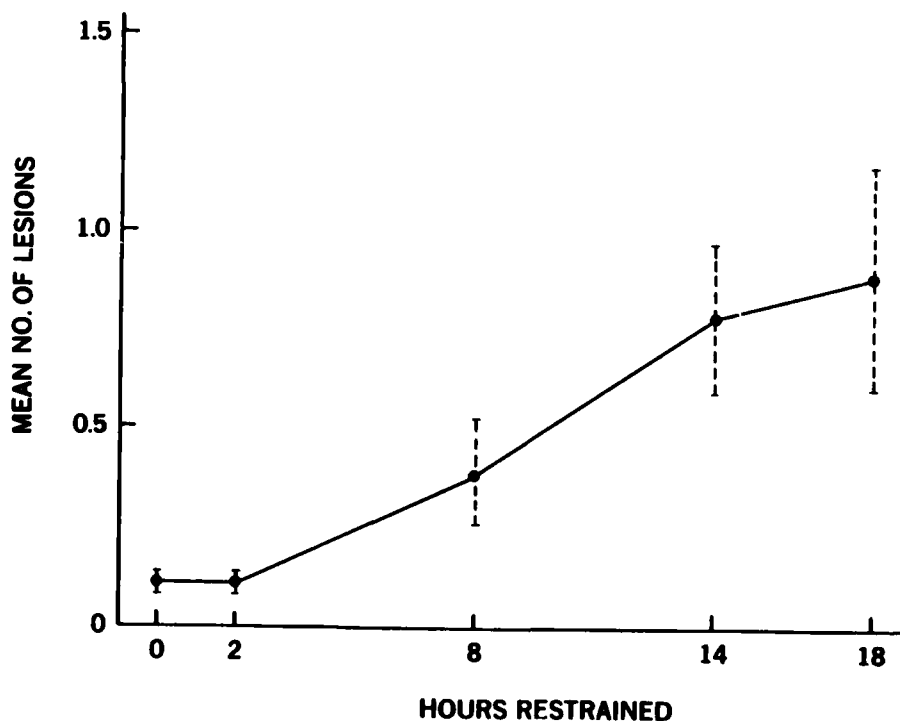


Figure 14. The mean number (± 1 S.D.) of stomach ulcers after 0, 2, 8, 14, and 18 hr of restraint stress.

Relationships Between Lesions and Behavior

A Spearman's correlation coefficient was obtained for comparison of the numbers of lesions and each of the behavioral measurements (5 categories of activity maze performance, numbers of avoidances, escapes, failures to respond, avoidance latencies, and number of responses to criterion). Each group (5 stress groups X 2 sexes = 10 groups) was ranked according to group mean in each behavioral category. Group rank in each behavioral category was then correlated with the group ranks for mean number of ulcers.

In general, a strong relationship existed between the behavioral measures and the presence of lesions (Table 2).

TABLE 2. CORRELATIONS AND ASSOCIATED STATISTICAL PROBABILITIES BETWEEN BEHAVIORAL RESPONSES AND THE NUMBER OF STOMACH LESIONS

Behavior	Correlation with ulcers	Probability
<u>Activity maze:</u>		
Latency to leave center square	$r_s = 0.2$	NS
Ambulations	$r_s = -0.57$	$p < .05$
Center square crossings	$r_s = -0.16$	NS
Rearings	$r_s = -0.61$	$p < .05$
Groomings	$r_s = 0.66$	$p < .05$
<u>Shuttle-box:</u>		
Failures to respond	$r_s = 0.82$	$p < .01$
Escapes	$r_s = -0.36$	NS
Avoidances	$r_s = -0.65$	$p < .05$
Latency after CS	$r_s = 0.75$	$p < .01$
Trials to criterion	$r_s = 0.90$	$p < .01$

In the activity maze, groups of animals with greater numbers of lesions showed reduced activity, with fewer ambulations, rearings, and more groomings. For animals in the shuttle-box, we noted more failures to respond, fewer avoidances, more trials to criterion, and longer overall response latencies.

DISCUSSION AND CONCLUSION

We have demonstrated stomach lesions, reduced activity, and decrements in avoidance learning--all induced by restraining rats in wire-screen tubes. The males showed greater decrements than females, especially at the shorter restraint times. Longer restraint periods resulted in greater performance decrements for both sexes, and the differences between sexes tended to disappear.

Direct adrenal and other hormonal manipulations have previously been shown to affect responses in an aversive conditioning situation. ACTH facilitates acquisition of an active avoidance response, and delays extinction of that response. Corticosterone facilitates extinction of an active avoidance response and inhibits passive avoidance. These somewhat opposed effects might be explained by operation of indirect pituitary-adrenal feedback mechanism. However, evidence exists that ACTH and the corticosteroids have direct effects on the central nervous system (12, 13). Park and Scarborough (30) showed that RES stimulation and depression had opposite effects on a conditioned emotional response to shock. RES stimulation attenuated conditioned suppression (decreased fear), whereas depressing the RES augmented the suppression (increased fear). An adrenalectomized and gonadectomized group of animals showed responses identical to the RES depressed group. Administration of an RES stimulant to this latter group restored responses to the normal level (30). Similarly, ionizing radiation, with its potent compromising effect on the immune system, produced more profound decrements in avoidance performance in males than in females. Mickley (25) has shown that hormone-influenced sex differences mediate these post-irradiation avoidance performance effects in the rat.

This study was unique in its combination of behavioral and physiological indices of environmental stress. Immobilization was chosen as an environmental stressor for rodents because we felt that potent changes could be produced without direct physical injury to the animal. Immobilization had previously been found to produce ulcers (15), and direct manipulation of the pituitary-adrenal "stress" hormones had been shown to affect avoidance conditioning (13). Previous researchers had not been able, however, to produce reliable two-way shuttle-box avoidance deficits in rodents following environmental stress (24).

The most commonly used stressor in the laboratory has been electric shock. In the typical learned helplessness procedure, animals were given a number of unpredictable shocks from which they could not escape. Dogs were subsequently unable to learn a simple escape-avoidance task--but rats did not differ reliably from controls in either escape or avoidance. Learned helplessness has only been demonstrated in rats with more difficult tasks, such as FR-2 escape. In that task, rats were required to: leave the first compartment of the shuttle-box (where the CS and shock were present); enter the second compartment (still in the presence of CS and shock); and return to the first compartment to terminate the shock. Relative to rats given an equal number of predictable shocks (22, 28, 29, 33), those rats given prior unsignaled shocks showed FR-2 escape deficits. Resorting to the complexity of the FR-2 procedure has been justified by arguments concerning the cognitive nature of learned helplessness (24).

Our experiment was both theoretically and procedurally simpler, and was therefore limited. By using immobilization rather than shock, we were able to produce performance deficits in simple two-way avoidance conditioning. Not only was the task simpler, but, because the original stressor was different from the shock encountered in the behavioral tests, we avoided the possible criticism that during the stress the animal learned a specific response that was incompatible with the response to be learned. That is, the noncontingent shock of Meier and Seligman (24) may teach the animal that responses will not be reinforced. Because of the consequent freezing, the animal is unlikely ever to make an escape or avoidance response and thus be exposed to the altered contingencies. Hence, we did not have to resort to intervening variables (such as learned helplessness) to explain our results, particularly in the light of the similar deficits shown by DeWeid et al. (13), after direct endocrine manipulation. We would point to the common element of stress in the two situations, and argue that restraint was a more effective stressor than unpredictable shock for the rat.

Although survival time was too short in our experiment to demonstrate the expected changes in adrenal weights, biochemical assays have shown elevated plasma epinephrine and norepinephrine as well as decreased adrenal epinephrine following immobilization (20, 21). Theorizing about the biological and evolutionary relevance of restraint (e.g., capture by a predator) is tempting. However, the empirical observation--that restraint procedures of various types are methodological favorites among stress researchers for the production of gastrointestinal lesions in rats--attests to the effectiveness of restraint as a stressor (15). In this regard, we are particularly gratified that our groups with greater numbers of lesions also showed the largest response decrements.

While responses in the open-field maze presumably reflect motivational changes, the shuttle-box avoidance task should reflect changes in motivation and learning capacity. The degree of motivational versus learning effects cannot be distinguished in the avoidance situation, where the animal's responses can change the contingencies to which he is exposed. Some theorists have argued that a failure to learn after stress is a motivational effect, a depression in activity produced by a stress-related depletion of norepinephrine (16). One may also reasonably assume that depletion of norepinephrine (or of the amines, in general) could contribute to reduced learning capacity (19). Approximately equal numbers of escape responses were noted in all groups. Even individual animals who failed to respond on a majority of trials generally had several escape responses early in the training session. This finding suggested that these individual animals simply did not derive the same cognitive associations from their responses. Similar experiments, using tasks (such as conditioned suppression) in which the animal's responses do not change the contingencies, are needed to clarify the issue.

Behavior may be the final common pathway which reflects the interaction of the many types of stressors which are of interest to the U.S. Air Force. Parallels can be drawn between our results with immobilization stress and those of Mickley (25) with ionizing radiation. He found in rats, after 10,000 rads of ionizing radiation, decrements which were greater in males than in females. Whaley, Scarborough, and Reichard (39) found a behavioral interaction between ionizing radiation and previous exposure to drum-trauma. Animals who had built up a tolerance to drum-trauma showed decreased saccharin

avoidance postirradiation. Whaley, Scarborough, and Reichard (39) proposed a nonspecific stress immunity that could provide a cross-resistance between many forms of trauma. Effects from different stressors, to which no tolerance had developed, would be expected to increase the severity of a performance decrement.

That performance may be reduced by the psychological effects of the stressing situation, as well as by direct physical insults, should be of great relevance in predicting responses in military emergencies. The results of this experiment show that, with an appropriate stressor, behavioral deficits and accompanying physiological changes can be produced in rats. This animal model may be appropriate for the military to use in economically determining similarities in response deficits to psychological and physical stressors likely to be encountered by combat personnel. The simplicity of this model may be particularly valuable for clarifying interactions between stressors. Without reference to intervening variables, our results support the assumption that stress has a generalized tendency to reduce activity and to interfere with avoidance acquisition.

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A P P E N D I X E S A - D :

- A. Software and Hardware Control for Rat Radiation and Stress Experiments
Under Protocol 7757-05-43D
- B. Terms Used in MANX Programs
- C. Program for Shuttle Escape and Avoidance Testing and Sample Data Sets
- D. Input-Output Look-up Table for Escape and Avoidance Testing System

APPENDIX A: SOFTWARE AND HARDWARE CONTROL FOR RAT RADIATION AND STRESS
EXPERIMENTS UNDER PROTOCOL 7757-05-43D

INTRODUCTION

Appendix A and the following three Appendixes (B, C, and D) document in detail the laboratory apparatus, computer interface, and computer programs used to implement experiments to measure the performance effects of exposure to ionizing radiation and/or psychological stress in rodents (rats). The general properties of the behavioral control computer package (MANX) used in this laboratory have already been documented (6).

For the computer to control the various aspects of the behavioral experiments, two-way communication was necessary between the computer and the laboratory behavioral apparatus. Computer outputs are in the form of high-speed, low-power transistor-to-transistor logic (TTL) pulses. The digital inputs required by the computer must also have TTL properties. On the other hand, the laboratory apparatus--with which the animals interact--was produced by various vendors, and was designed with various power requirements. Response-produced signals, typically switch closures, have some undesirable electrical characteristics (e.g., contact bounce) that necessitate conditioning of the signals before they can serve as appropriate computer inputs. These factors introduced various requirements for interfacing between the computer and the experimental apparatus.

Computer programs specified the sequence of events to take place in each test session, and defined the contingent relationships between the responses of the subjects and the stimuli presented. Programs likewise performed some data processing as the data were acquired, in order to provide the experimenter with immediate feedback on the progress of the experiment. Under program control, the computer also stored a detailed data set from each testing session for later analysis. Direct data acquisition by the computer greatly reduced the effort required later for data entry to computer systems that provided statistical analyses of results.

In the present experiments, testing was conducted during the acquisition of shuttle escape and avoidance behavior.

In the shuttle escape and avoidance paradigm, the rat was tested in a two-chambered test apparatus. Each of the two chambers was equipped with identical house lights, overhead signal lights, and grid floors. A partition with a door (open during shuttle avoidance testing) separated the two

EDITOR'S NOTE: The reference numbers which appear in Appendix A are keyed to the list of References on pp. 28 - 31.

--APPENDIX A--

chambers. The grid floor of each chamber was suspended in such a way that the presence of a rat's weight in the chamber produced a slight movement of the floor that was detected by the closure of a switch. The closures of the two switches provided response indicators in this task. The stimuli controlled by the computer were--conditioned stimulus (CS): overhead light flashing at 2 Hz, 50% duty cycle; unconditioned stimulus (US): foot shock of approximately 0.2 mA, 2 Hz (synchronous with CS), 10% duty cycle; and house light: a dim pilot light mounted on the end wall of each chamber, continuously lit except during the CS when the light flashed in synchrony with the CS. The house light was extinguished at the end of each test session. When the CS was presented, the rat could avoid the US by crossing to the other chamber (shuttle response). If no shuttle response occurred during a 5-sec CS presentation, the US was also presented. The rat could then make a shuttle response to escape the US. Both CS and US (if present) were terminated by the shuttle response.

To summarize--the instrumentation tasks to be accomplished, in order to provide computer automation of the testing paradigm specified in the experimental protocol, included:

- a. design and construction of testing chambers;
- b. interfacing the computer to the testing chambers so that the computer could sense responses and control stimuli; and
- c. developing computer programs to produce the stimulus-response contingencies required by the paradigm and to record the data generated by each subject.

APPARATUS

Experimental chambers (Fig. A-1) were so designed and constructed as to serve both as shuttle-boxes for escape and/or avoidance testing, and as Skinner boxes for other experiments. A shocker-distributor (Coulbourn Instruments Model E13-16) was connected to the grid floor on each side of each chamber. Pilot lights, mounted on the walls at each end of the chamber, served as house lights. Lights mounted above the translucent overhead panel on each side of the chamber served as the CS. Openings in the wall at each end of the chamber for response levers and liquid reinforcement dippers were covered during the shuttle-box avoidance testing.

CONTROL APPARATUS AND COMPUTER INTERFACE

Illustrated in Figure A-2 are the locations and interconnections among the major system components involved in these experiments. The computer, with its resident digital input-output (I/O) cards, was located in a room separate from the animal-testing rooms. Ribbon cables and a communications line connected the computer to the MANX interface panels, the Digibit logic elements, and a control console located in another room, where monkeys were

--APPENDIX A--



Figure A-1. Shuttle-box for escape and avoidance testing.

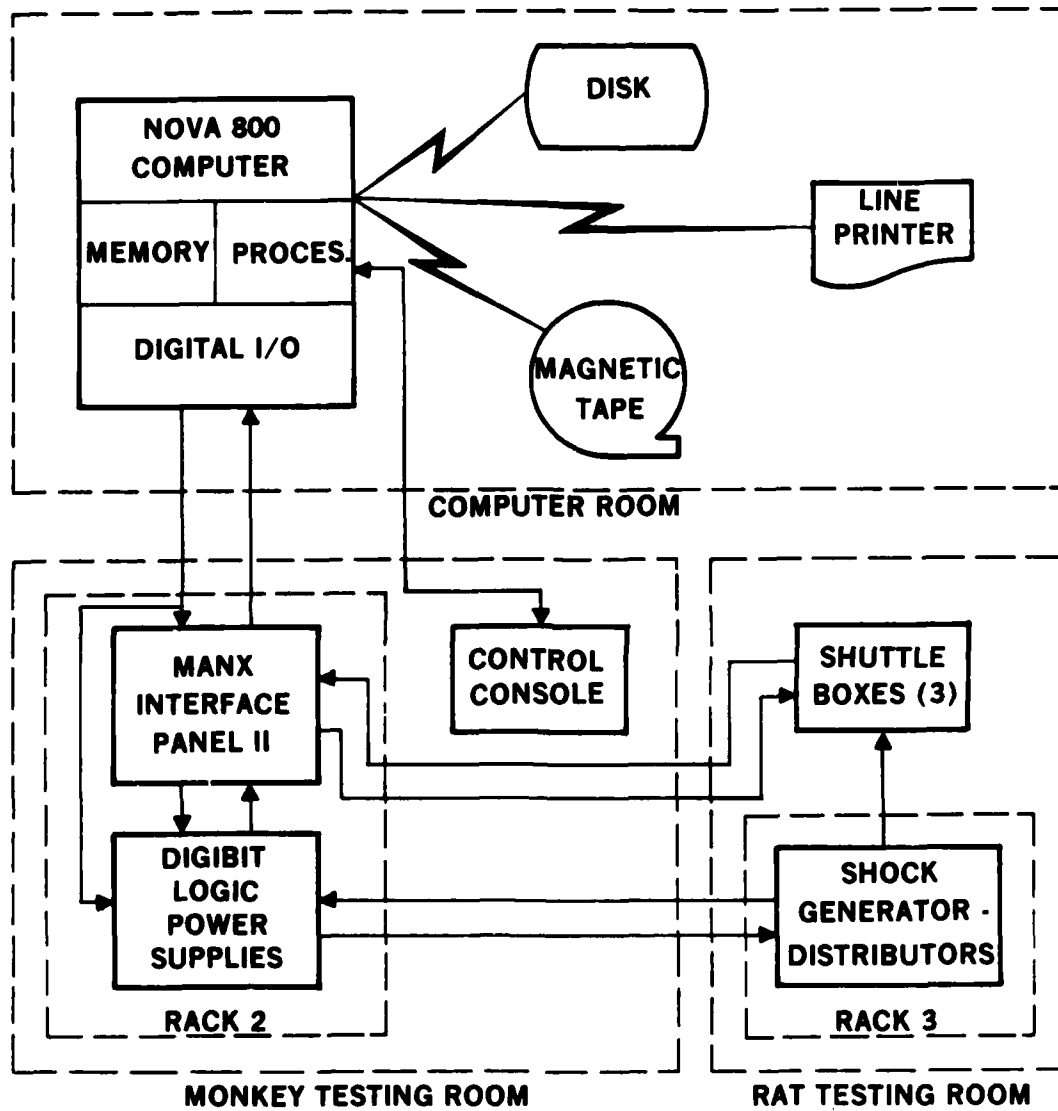


Figure A-2. Components of automated experimental control and data acquisition system.

--APPENDIX A--

tested. The MANX and Digibit interface elements were connected, via six extension cables (one for each side of each shuttle-box), to the rat experimental chambers in the rat testing room. Computer sensing of responses and control of stimuli were achieved via the MANX interface panel and the digital I/O card that was mounted in the NOVA 800 main frame. Both the MANX interface and the I/O card were purchased from General Controls, Inc., of Smithville Flats, New York. The MANX panel served as a set of buffer amplifiers between the I/O card and the experimental apparatus, converting response inputs to TTL pulses and converting computer output signals (TTL) to 28-V signals to drive stimulus devices.

Two kinds of shocker-scrambler units have been used in the laboratory. Each required control signals of a different voltage. To achieve flexibility, an auxiliary Digibit interface (Fig. A-3) was assembled. The Digibit logic units were so configured as to provide -12 V control signals for shocker-scrambler units, manufactured by BRS-Foringer (BRS), or +28-v control signals for the Coulbourn Instruments Model EI3-16 Shocker-Distributors used for these experiments. The ribbon-cable (G), connecting 16 output bits of the digital I/O board in slot 11 of the computer to the upper 16 output channels of the second MANX interface panel, was tapped a few inches behind the panel. A short section of ribbon cable was used to connect 6 of the lines in cable G to the inputs of Digibit Positive to Negative Logic Converters (PN-252), which converted the TTL positive logic outputs of the I/O board to Digibit (-12-V) logic. PN-252 outputs were then inverted by Digibit Inverters (IN-201). The inverted pulses caused contact closures in Reed relays (RY-204). These contact closures provided driving signals to the shock-distributors. Driving signals appropriate to the Coulbourn shockers (+28-V) were arranged by connecting the relay contacts to the appropriate supply voltage. The Digibit units plug in to back-wired connectors mounted in a standard Digibit rack. The position of each unit in this rack (Fig. A-3) is indicated by the number (A1-A9) of its connector. Letters shown by each input and output of a Digibit unit refer to the pin labels on the back-wired connectors. All other interfacing requirements were met by the MANX interface.

Shown in Figure A-4 are the details of connections between the shuttle-boxes and the interface panel. The devices on each side of the three identical shuttle-boxes were wired similarly to a Cinch-Jones 25 connector plug (P2) mounted at the back of the shuttle-box base. For each side, an extension cable provided connection to a mating Cinch-Jones plug (P1) mounted on the back of Rack 2. Wires in the extension cables and in the cables from P1 to MANX interface terminal strips are similarly color-coded. Wires connected to one side of one shuttle-box (3L for box 3, left side) can be traced from the box to the connector (P2), through the extension cable to P1, and finally to terminals in Rack 2. Except for color code, box 3, right side (3R), was similarly cabled. The cables, that formed the extension cables and the connections to the MANX interface, contained wires in twisted pairs. While a given color of wire could occur several times in such a cable, that wire was never paired with the same color twice.

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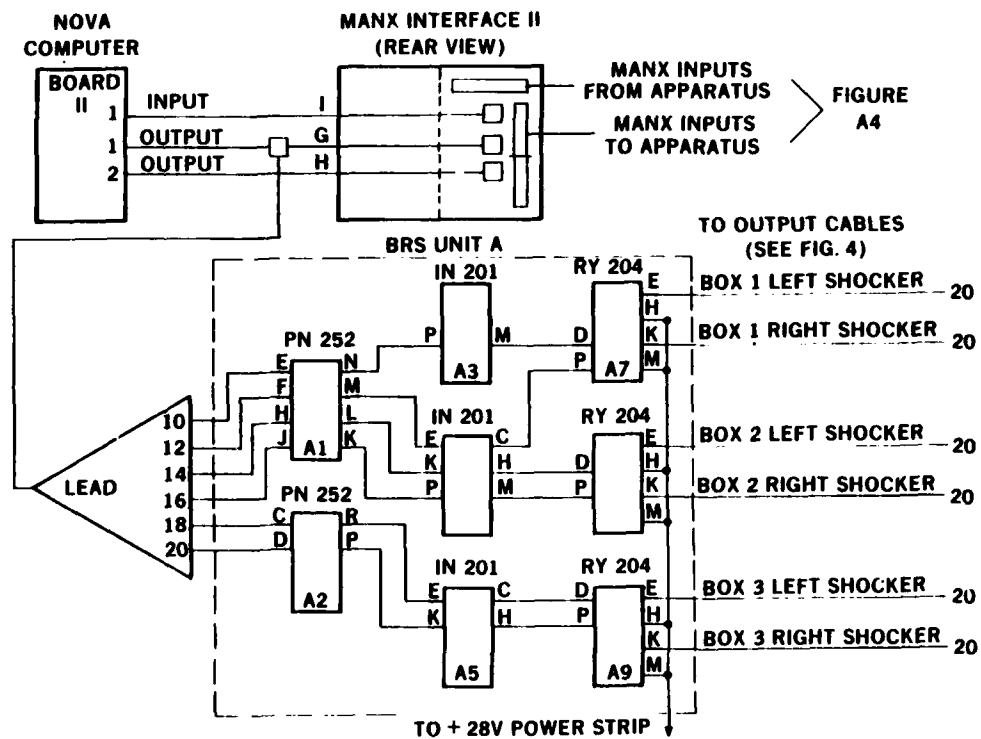


Figure A-3. Auxiliary interface for control of shocker-scrambler units.

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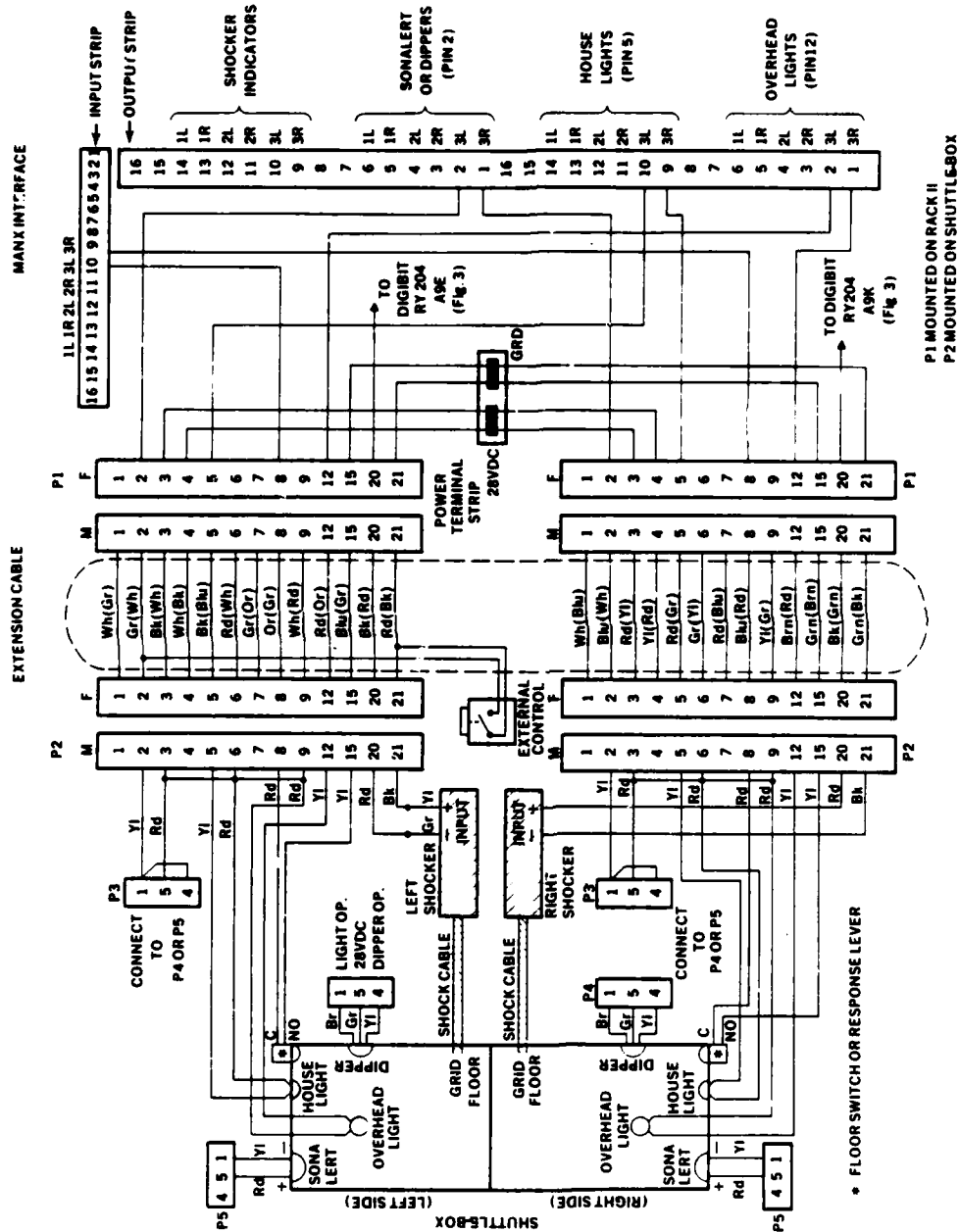


Figure A-4. Circuit diagram showing connections between the shuttle-boxes and the interface panel.

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A black wire paired with a white one could thus be uniquely identified as black (white). The cable from each side of each shuttle-box was connected to a different set of terminals on the back of the MANX interface panel. (These connections are shown schematically at the right of Figure A-4.)

COMPUTER CONTROL PROGRAMS

Each animal was trained or tested by being placed in a shuttle-box behavioral station for which the appropriate MANX binary program had been loaded. The general properties of the MANX software system and the MANX programming language (State Notation) have already been described (6). Appendix B contains a description of the notation and functions of this language. Appendix C contains the program used for shuttle-box avoidance testing. Complete data gathered from a representative animal are also included, to illustrate the detail of the raw data set and the measures derived from it for analysis.

The program (Appendix C) started a 5-min adaptation period when the rat was placed in the chamber. During this period, the house lights at the ends of the chamber provided the only externally controlled stimuli. The program monitored the rat's position, but no response-contingent stimuli were presented. At the end of the adaptation interval, a series of 60 escape-avoidance trials was presented, with intertrial intervals varying at random from 30 to 60 sec. The mean intertrial interval was approximately 45 sec. Three sec before the beginning of each trial, response input lines were tested to ascertain that only one floor switch was actuated. If both were actuated (as would be the case if the animal were standing in the opening between the two sides), a brief shock was delivered to the grid floors on both sides and the test was repeated 7 sec later. At the end of the intertrial interval (and after the foregoing test showed the animal to be occupying only one side) the CS was presented on that side. The CS consisted of both the bright overhead light and the dim house light flashing at 2 Hz (50% duty cycle). The CS terminated whenever the animal crossed to the other side. After 5 sec without a response, the US (foot shock) was delivered. CS and US continued until either the animal crossed to the other side, or 15 sec elapsed without a shuttle response.

Response latencies were recorded in two ways. Whenever a shuttle response to the CS or US occurred, a count was added to the appropriate 1.0-sec-wide bin of a response latency histogram. Response latencies were also encoded in the disk data set as interevent times, with temporal resolution accurate to .01 sec. Counters were used to accumulate the number of trials required for the animal to meet several response criteria: 3 avoidances, 10 escapes, and 8 avoidances in any 10 or fewer consecutive trials. After each block of 10 trials, the total number of escapes and avoidances were written to the disk data set.

At the end of each animal's run, the values of the program counters were output to the console or line printer (refer to Appendix C, for example),

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which provided immediate data on each animal's performance that were sufficient for routine analyses. At the end of each testing day, the datasets for every animal were decoded and printed (refer to Appendix C, for example) to insure the possibility of analyzing the data independent of the laboratory computer. After each group of animals was tested, the datasets for the group were processed to derive group data to be entered into statistical analyses via other computers. For each block of 10 trials for each animal, processing on the laboratory computer provided the mean, median, standard deviation, and semi-interquartile range of the response latencies. These data from one group of animals are illustrated in Appendix C.

The program illustrated in Appendix C accepted inputs (e.g., RI) from, and sent outputs (e.g., ON 2--turn on stimulus 2) to, the behavioral stations. Therefore, the MANX system needed to "know" which of the 64 input and 128 output lines were associated with the respective stimuli and responses in the respective stations. This information was stored in a table that the MANX Run Time System used to "look up" the information. Thus, when a program loaded in Station 6 contained the output "ON 3," the Run-Time System went to a look-up table stored in the disk (usually named MANX.IO) to determine which output line was to be activated in order to produce stimulus 3 in Station 6. An annotated example of the look-up table used for these experiments is presented in Appendix D.

In summary, Appendixes A-D document (as required by Form DD 1423) the apparatus and programs used to gather the data for this Technical Report.

APPENDIX B: TERMS USED IN MANX PROGRAMS

A MANX program consists of one or more "State Sets," each containing several related states. The states of each State Set are related in that every state (except, possibly, the first) can be entered from at least one other state of the set. The first state of each State Set is entered when the program is loaded to a testing station. During transitions from state to state, stimulus conditions in the station can be changed. Transitions occur when specified input conditions are met. Each MANX program statement thus has the following format:

Input Condition: Operations transition to→ Destination State

Input Conditions (or contingencies) that can be specified in MANX programs include:

- 1) Passage of time (e.g., 5": or AT:, where A is a variable that can be modified by the program).
- 2) Occurrence of one or a specified number of response events (e.g., RI:, 1OR2:, or BR3:, where B is a program variable).
- 3) Occurrence of events generated as output operations by other State Sets or by other states within the same State Set. This special class of events (Z pulses) provides a means of coordinating output operations of different State Sets and of gating outputs and transitions within a State Set. For example, 10Z1: is a condition in one State Set that is satisfied when an operation of another State Set has occurred 10 times. Z2 & A(X): is a condition satisfied only when both a Z2 pulse occurs and the variable A has a value equal to X.
- 4) Logical AND or OR Gates -- Each of the three types of input conditions (just specified) can be combined with the value of a program variable or with a probability value to form a compound (AND) condition. Examples:

Z1 & A(X): Both Z1 and A = X.

1OR2 & P(500): Satisfied with probability $\frac{1}{2}$ after 10 R2 responses occur.

5" & B(1): Satisfied after the passage of 5" only if B = 1.

Conditions listed separately within a single State are combined in logical OR fashion. The compound:

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Z1 & A(2): ----> S2

Z1 & A(3): ----> S3

Z1 & A(4): ----> S4

: ----> S1

produces transition to one of four states whenever a Z1 pulse occurs, depending on the value of variable A. Transition to state 2, 3, or 4 occurs when A = 2, 3, or 4, respectively. Otherwise, transition to state 1 occurs. Probability OR-gating is accomplished similarly.

Summary of Input Conditions

<u>Condition:</u>	<u>After entry to the state, transition occurs:</u>
R1:	When Response 1 occurs.
10 R1:	On the 10th response.
AZ1:	When a number of Z1 pulses has occurred. The number equals the value of A on entry to the state.
7.52":	When 7.52 sec have elapsed.
50Z2: 45':	When 50Z2 pulses have occurred, OR 45 min have elapsed.
R1 & B(5): & B(6): & B(7):	When R1 occurs, only if variable B = 5 OR 6 OR 7. (Each condition may be associated with a different destination state).
Z15 & P(333): ----> S2 & P(333): ----> S3 : ----> S4	On Z15, to state 2 OR 3 OR 4 with equal probability.
1" & B(2) : ----> S10 & P(500) : ----> S2 : ----> S3	After 1 sec, to state 10--only if B = 2; otherwise, to state 2 OR 3 with equal probability.

From a state, transition can occur to any state in the State Set, including the originating state. Recurrent transitions (from a state to itself) can take two forms: null transition, and normal recurrent transition. Null transition is denoted by: e.g.,

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S1, (label for the state)

R1:Z1 ----> SX (null transition)
1':Z2 ----> S2
10Z3:Set A=10 ---->S3.

Normal recurrent transition is denoted by:

S1,

R1:Z1 ----> S1 (recurrent, but normal)
1'':Z2 ----> S2
10Z3:Set A=10 ----> S3

Null transition generates the output operation (Z1) without changing the timer and counter contingencies in the state. Thus, in the first example, transition to state 2 will occur after 1 min, or transition to state 3 will occur on the tenth Z3 pulse (whichever condition is satisfied first), independent of the occurrence or nonoccurrence of response 1. In the second example, whenever response one occurs, the 1-min time contingency and the Z3 counter (10) are reset. Thus, transition to state 2 will occur only if 1 min elapses without a response; transition to state 3 will occur only if no response occurs and 1 min does not elapse before 10 Z3 pulses are generated. The two types of recurrent transitions can thus be used to generate very different contingencies.

Output Operations

Whenever an input condition is satisfied and transition occurs, any number of output operations can occur. Output operations can take 6 forms:

- a) Turn on stimuli (e.g., ON 3).
- b) Turn off stimuli (OFF 7).
- c) Generate Z pulses (Z1).
- d) Change values of program variables (ADD A; SUB B; Set C=2, DT=3', E=B+C, D=E*A, etc.; ADD A is equivalent to SET A=A+1; SUB B is equivalent to SET B=B-1).
- e) Increment counters in the counter array for the station (C1; CA, where variable A points to the appropriate counter).
- f) Perform a MANX function.

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MANX functions include:

<u>Function</u>	<u>Operation</u>
CODE N	Store code N in the disk dataset, along with the time on the station timer, and reset timer to zero; i.e., store an event code and the interevent time.
SPCOD N,X	Store two codes, N(0-63) and X(0-31), with the interevent time.
DUMP	Write the counter array values in the dataset; reset counters.
LIST N,A,V,N ₁ ,N ₂ ,...,N _N	Set variable V equal to N _A . Increment A. If A=N+1, set A=1.
RAND N,A,V,N ₁ ,N ₂ ,...,N _N	Set variable V equal to one of the arguments (N ₁ -N _N) determined randomly. Repeated sampling is without replacement. Variable A is set equal to N, initially, and is used to restart when all arguments are exhausted.
TIME X,Y,Z	Set X=time of day in: hours only (if Z is omitted); minutes only (if Y is omitted); or hours and minutes. At 3:15 P.M., X=1515.
TYPE "TEXT",X,Y,"MESSAGE"	Type the arguments (character strings or variable values) on the system console.
WRITE N,V	Like CODE N, except that the value of variable V is written instead of an interevent time.
CALL HTDIO(9,A,B,C)	Set a 16 bit digital I/O (device code =C). A and B specify the most and least significant 8 bits, respectively.
CALL AD(N,V,CH)	Convert analog signal on channel CH to digital value V.
CALL TEST(11,A,B,C)	Set C = 1 if A>B, C = 2 if A=B, C = 3 if A<B. A and B may be constants or variables (numeric or time).

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CALL DA(15,A,CH,B)

On digital-to-analog card B, output analog signal on channel CH with value proportional to variable A.

CALL STANO(17,A,B,C)

Set A = station number
B = subject number
C = program number

CALL PTCNT(18,A)

Print header information and counter array on:
system console if A = 1,
second console if A = 2,
line printer if A = 3.

MANX system users can define and use other functions tailored to meet their specific needs.

The preceding description of MANX programming terms is intended as an aid to the interpretation of the program presented in the Appendix C.

APPENDIX C: PROGRAM FOR SHUTTLE ESCAPE AND AVOIDANCE TESTING AND SAMPLE DATA SETS

```

/SHUTTLE.SK -- PROGRAMMER: D. W. BLICK
/SOURCE CODE FOR PROGRAM TO RUN 60 SHUTTLE-BOX AVOIDANCE
/TRIALS WITH MEAN INTERTRIAL INTERVAL 45"

/COUNTERS: 1-15 ARE RESPONSE LATENCY HISTOGRAM BINS, 1"
/16- LATENCIES > 15"
/17- TRIALS TERMINATED WITHOUT RESPONSE
/18- AVOIDANCES
/19- ESCAPES
/20- TRIALS TO 3 AVOIDS
/21- TRIALS TO 10 ESCAPES
/22- TRIALS TO CRITERION (AT LEAST 8 AVOIDS IN 10 TRIALS)

/CNTS=22
/PGMNO=7

/VARIABLES
/A - RAND INTERVAL INDEX
/B - FLAG FOR CS LOCATION
/C - # OF AVOIDS SINCE LAST SHOCK
/D - # OF AVOIDS SINCE PREVIOUS SHOCK
/E - TEST VALUE FOR C,C+D
/F - BIN FOR LATENCY HISTOGRAM
/G - # OF AVOIDS
/H - # OF ESCAPES
/I,J- TEST VALUES FOR G,H
/RT- ADAPTATION INTERVAL
/ST- CS-CS INTERVAL
/W - FLAG FOR POSITION OF RAT

STIMULI
S1 - LEFT CS
S2 - RIGHT CS
S3 - LEFT HOUSE LIGHT
S4 - RIGHT " "
S5 - LEFT SHOCKER
S6 - RIGHT SHOCKER

/Z-PULSES
/1 - INTERNAL
/2 - START CS
/3 - END OF TRIAL
/4 - AVOIDANCE
/5 - FAILURE TO AVOID
/6 - START SHOCK
/8 - AVOIDANCE OR ESCAPE RESPONSE
/10- END SESSION AFTER 60 TRIALS
/11- RAT IN BOX
/12- START STATION
/13- START CS

RESPONSES
R1 - ENTER LEFT BOX
R2 - " RIGHT "
R3 - START STATION
R17- " "
R18- STOP STATION

/CODES AS PER RATLABEL.DC, I/O AS PER SHUTTLE.IO

S.S.1. /INITIALIZE VARIABLES, TIME ADAPTATION

S1. /WAIT FOR START
Z12:---->S2
S2. /WAIT FOR RAT, DETECT RESPONSES
R1:SET W=1,Z11:---->S3
R2:SET W=2,Z11:---->S3

S3. /INITIALIZE, WRITE SESSION START TIME
/TO DATA SET, ZERO STATION TIMER
Z11:SET RT=5,A=7,B=0,C=0,D=0,G=0,H=0,L=0,
TIME X,Y,Z;WRITE 62,X,CODE 64:---->S4
R1 & W(2) SET W=1,CODE 20:---->SX
R2 & W(1) SET W=2,CODE 25:---->SX
RT:Z13:---->S5

```

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```

S5.          /KEEP TRACK OF POSITION, PROVIDE RESPONSE
              /INFORMATION (Z8) TO OTHER STATE SETS

R1 & W(2) SET W=1; Z8---->SX
R2 & W(1) SET W=2; Z8---->SX

S. S. 2.          /TRIAL TIMER

S1.          /SELECT CS-CS INTERVAL
              Z13 & B(3); Z10---->S6
              RAND7; A; ST; 27"; 32"; 37"; 42"; 47"; 52"; 57"---->S2
S2.          /WAIT FOR CS-CS INT. (LESS 3 SEC.)
              Z10:---->S6
              ST:---->S3
S3.          /TEST FOR STANDING IN BOTH SIDES
              3"; Z13---->S1          /OK, START TRIAL
              Z28: ON 5; ON 6; CODE 28---->S4
S4.          /SHOCK BOTH SIDES
              10" OFF 5; OFF 6---->S5
S5.          /RECHECK
              7"---->S3
S6.          /STOP
              R18:---->SX

S. S. 3.          /PRESENT CS

S1.          /QUIT OR PRESENT CS
              Z13 & B(3) OFF 3; OFF 4---->S7
              Z1---->S2
S2.          /START A SIDE DEPENDING ON POSITION
              Z1 & W(1); ON 1; ON 7; CODE 40; SET B=1; Z2 ---->S3
              Z1 & W(2); ON 2; ON 8; CODE 41; SET B=2; Z2---->S5
S3.          /LEFT SIDE, CYCLE @ 2 HZ 'TIL RESPONSE
              Z3: OFF 1; OFF 7; ON 3; Z1---->S1
              25" OFF 1; OFF 7; OFF 3---->S4
S4.          Z3: ON 3; Z1---->S1
              25" ON 1; ON 7; ON 3---->S3
S5.          /RIGHT SIDE, CYCLE @ 2 HZ 'TIL RESPONSE
              Z3: OFF 2; OFF 8; ON 4; Z1---->S1
              25" OFF 2; OFF 8; OFF 4---->S6
S6.          Z3: ON 4; Z1---->S1
              25" ON 2; ON 8; ON 4---->S5
S7.          /STOP
              R18:---->SX

S. S. 4.          /AVOID, ESCAPE DETECTION, HISTO COUNT

S1.          /BRANCH FOR CODING DEPENDING ON WHERE CS IS
              Z2 & B(1) ---->S2
              Z2 & B(2) ---->S4
S2.          /CS LEFT, DETECT & CODE AVOIDANCES
              Z8: C18; CF; ADD G; CODE 26; Z4; Z3---->S1          /AVOID
              5"; Z6---->S3          /NO RESP., UCS ON
S3.          /DETECT & CODE ESCAPES, FAILURES TO RESPOND
              Z8: C19; CF; ADD H; CODE 27; Z5; Z3---->S1          /ESCAPE
              15" C17; CODE 31; Z5; Z3---->S1          /FAILURE
S4.          /CS RIGHT, SAME AS ABOVE
              Z8: C18; CF; ADD G; CODE 21; Z4; Z3---->S1
              5"; Z6---->S5
S5.          Z8: C19; CF; ADD H; CODE 22; Z5; Z3---->S1
              15" C17; CODE 31; Z3---->S1
              ---->S1

```

--APPENDIX C--

```

S. S. 5,          /HISTO BIN COUNTER

S1,              /INITIALIZE AT CS START
Z2: SET F=1---->S2

S2,              /ADVANCE POINTER EACH SECOND
Z3: ---->S1
1": ADD F; Z1---->S3

S3,              /CHECK FOR LATENCY > 15"
Z3: ---->S1
Z1 & F(16): ---->S1
: ---->S2

S. S. 6,          /SHOCKER CONTROL

S1,              /SHOCK AND CODE DEPENDING ON CS SIDE
Z6 & B(1): ON 5; CODE 30---->S3
: ON 6; CODE 35---->S5

S2,              /LEFT SIDE, CYCLE @ 2 HZ 'TIL TRIAL ENDS
Z3: OFF 5---->S1
.45": ON 5---->S3

S3,              .05": OFF 5---->S2
Z3: OFF 5---->S1

S4,              /RIGHT SIDE, CYCLE @ 2 HZ
Z3: OFF 6---->S1
.45": ON 6---->S5

S5,              .05": OFF 6---->S4
Z3: OFF 6---->S1

S. S. 7,          /COUNT TRIALS TO 3 AVOIDS
S1,              /AT START OF TRIAL, COUNT TRIAL, TEST # OF AVOIDS
Z2: C20; CALL TEST(11, 3, G, I); Z1---->S2
S2,              /IF # OF AVOIDS >= 3, STOP, ELSE CONTINUE
Z1 & I(2): ---->S3
Z1 & I(3): ---->S3
: ---->S1

S3,              R18: ---->SX

S. S. 8,          /STATION START, STOP

S1,              /START ON SWITCH (R3) OR KEYBOARD R17
R3: ON 3; ON 4; Z12---->SX
R17: ON 3; ON 4; Z12---->SX
Z11: ---->S2

S2,              /STOP ON R18 OR AFTER 60 TRIALS,
: /EVERY 10 TRIALS WRITE # OF AVOIDS, ESCAPES
60Z2: SET B=3---->S3
R18: TIME X, Y, Z; WRITE 63, X;
: OFF 3; OFF 4; OFF 5; OFF 6; DUMP---->STOP
10Z2: WRITE 51, G; WRITE 52, H---->SX

S3,              /END OF 60TH TRIAL, PRINT COUNTERS TO LINE PRINTER
Z3: TIME X, Y, Z; WRITE 63, X; OFF 3; OFF 4; OFF 5; OFF 6;
CALL STANO(17, K, L, M); CALL PTCNT(18, 3)---->S4

S4,              2": TYPE "RAT #", L, " IN STATION", K, " DONE, ";
: TYPE "R18 TO SAVE DATA AND STOP!"---->S5

S5,              R18: DUMP---->STOP

S. S. 9,          /COUNT TRIALS TO 10 ESCAPES
S1,              /COUNT EACH TRIAL, TEST # OF ESCAPES
Z2: C21; CALL TEST(11, 10, H, J); Z1---->S2
S2,              /IF # OF ESCAPES >= 10 STOP, ELSE CONTINUE
Z1 & J(2): ---->S3
Z1 & J(3): ---->S3
: ---->S1

```

--APPENDIX C--

```

S3.      R18: ---->STOP

S. S 10.      /COUNT TRIALS TO CRITERION,
              /C= # OF TRIALS SINCE LAST SHOCK,
              /D= # OF TRIALS SINCE PREVIOUS SHOCK
S1.      /COUNT TRIALS(Z2), PROCESS SHOCKS(Z5) AND AVOIDS(Z4)
Z2: C22---->SX
Z5: SET D=C, C=0---->SX
Z4: ADD C, CALL TEST(11, C, 7, E); Z1---->S2
S2.      /IF C>7 STOP, ELSE TEST C+D
Z1 & E(1): ---->S4
          /SET F=C+D; CALL TEST(11, F, 7, E); Z1---->S3
S3.      /IF C+D > 7 STOP, ELSE RETURN
Z1 & E(1): ---->S4
          /---->S1
S4.      R18: ---->STOP

$          /END OF PROGRAM

```

Sample of data displayed on the computer console and printed at the end of each test session. Date, time, and disk space availability are presented first:

```

5/13/82 6:11:33
LEFT: 1670   USED: 3194
20 64 0
4
24
31
1 4 6 10 2 10 4 2
0 1 2 0 0 0 0 0
0 0 0 0 0 0 0 0
18 23 19 28 31 47 60

```

Then experiment number (20), subject number (64) and group (0) are printed. The next three rows are station number (4), program number (24) and number of program counters (31). The next three rows are the first 24 program counters, which correspond to sequential one-sec wide bins of a response latency frequency histogram. The last row of program counters shows failures to respond (18), avoidances (23), escapes (19), number of trials to 3 avoidances (28), number of trials to 10 escapes (31), number of trials to a criterion of eight avoidances within 10 or fewer consecutive trials (47), and, finally, total number of trials (60).

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Sample of printout of complete data set (codes and inter-event times)
for one subject's 60-trial test session:

```
*****
SUBJECT NO :      3                      DATE:   8/20/81
EXP. NO.    GROUP NO.  STA NO.  PROG. NO.  NO. COUNTERS
  0          1          6         7         22
FILE NAME    S00082081.03
*****
```

CODE	TIME	LABEL
40	19.68	CS LEFT SH. BOX
27	19.02	ESCAPE TO RIGHT SH. BOX
41	15.98	CS RIGHT SH. BOX
22	9.58	ESCAPE TO LEFT SH. BOX
40	30.42	CS LEFT SH. BOX
27	6.33	ESCAPE TO RIGHT SH. BOX
41	23.67	CS RIGHT SH. BOX
22	9.21	ESCAPE TO LEFT SH. BOX
41	26.66	CS RIGHT SH. BOX
21	4.47	AVOID TO LEFT SH. BOX
40	40.53	CS LEFT SH. BOX
27	9.59	ESCAPE TO RIGHT SH. BOX
41	40.41	CS RIGHT SH. BOX
22	15.83	ESCAPE TO LEFT SH. BOX
40	9.17	CS LEFT SH. BOX
27	7.66	ESCAPE TO RIGHT SH. BOX
41	42.34	CS RIGHT SH. BOX
22	15.85	ESCAPE TO LEFT SH. BOX
41	3.54	CS RIGHT SH. BOX
51	0.01	END OF 10-TRIAL BLOCK
22	13.44	ESCAPE TO LEFT SH. BOX
40	16.56	CS LEFT SH. BOX
27	8.12	ESCAPE TO RIGHT SH. BOX
41	16.88	CS RIGHT SH. BOX
22	5.70	ESCAPE TO LEFT SH. BOX
41	19.01	CS RIGHT SH. BOX
22	6.38	ESCAPE TO LEFT SH. BOX
40	33.62	CS LEFT SH. BOX
27	5.95	ESCAPE TO RIGHT SH. BOX
41	49.05	CS RIGHT SH. BOX
22	5.71	ESCAPE TO LEFT SH. BOX
41	21.59	CS RIGHT SH. BOX
22	6.05	ESCAPE TO LEFT SH. BOX
40	38.95	CS LEFT SH. BOX
26	3.18	AVOID TO RIGHT SH. BOX
41	31.61	CS RIGHT SH. BOX
22	5.70	ESCAPE TO LEFT SH. BOX
41	18.42	CS RIGHT SH. BOX
21	3.28	AVOID TO LEFT SH. BOX
40	35.60	CS LEFT SH. BOX
51	0.03	END OF 10-TRIAL BLOCK
26	4.93	AVOID TO RIGHT SH. BOX
41	20.07	CS RIGHT SH. BOX
22	6.20	ESCAPE TO LEFT SH. BOX
40	23.80	CS LEFT SH. BOX
27	6.21	ESCAPE TO RIGHT SH. BOX
41	28.79	CS RIGHT SH. BOX
22	6.67	ESCAPE TO LEFT SH. BOX
40	38.33	CS LEFT SH. BOX
27	5.22	ESCAPE TO RIGHT SH. BOX
40	23.00	CS LEFT SH. BOX
26	2.36	AVOID TO RIGHT SH. BOX
41	37.59	CS RIGHT SH. BOX
22	5.81	ESCAPE TO LEFT SH. BOX
40	49.19	CS LEFT SH. BOX
27	6.32	ESCAPE TO RIGHT SH. BOX

--APPENDIX C--

41	23.68	CS RIGHT SH. BOX
22	5.95	ESCAPE TO LEFT SH. BOX
40	19.05	CS LEFT SH. BOX
26	4.58	AVOID TO RIGHT SH. BOX
41	45.42	CS RIGHT SH. BOX
51	0.06	END OF 10-TRIAL BLOCK
21	0.12	AVOID TO LEFT SH. BOX
40	33.55	CS LEFT SH. BOX
26	3.62	AVOID TO RIGHT SH. BOX
41	36.38	CS RIGHT SH. BOX
22	7.04	ESCAPE TO LEFT SH. BOX
40	22.96	CS LEFT SH. BOX
27	6.72	ESCAPE TO RIGHT SH. BOX
41	38.28	CS RIGHT SH. BOX
22	5.70	ESCAPE TO LEFT SH. BOX
40	49.30	CS LEFT SH. BOX
26	1.93	AVOID TO RIGHT SH. BOX
41	23.07	CS RIGHT SH. BOX
21	4.43	AVOID TO LEFT SH. BOX
40	20.57	CS LEFT SH. BOX
27	5.85	ESCAPE TO RIGHT SH. BOX
41	39.15	CS RIGHT SH. BOX
22	6.29	ESCAPE TO LEFT SH. BOX
40	28.71	CS LEFT SH. BOX
26	1.95	AVOID TO RIGHT SH. BOX
40	8.55	CS LEFT SH. BOX
51	0.11	END OF 10-TRIAL BLOCK
26	3.20	AVOID TO RIGHT SH. BOX
41	36.55	CS RIGHT SH. BOX
21	4.81	AVOID TO LEFT SH. BOX
40	50.19	CS LEFT SH. BOX
26	1.95	AVOID TO RIGHT SH. BOX
41	28.05	CS RIGHT SH. BOX
22	5.40	ESCAPE TO LEFT SH. BOX
40	28.53	CS LEFT SH. BOX
26	2.03	AVOID TO RIGHT SH. BOX
40	12.17	CS LEFT SH. BOX
26	2.79	AVOID TO RIGHT SH. BOX
41	42.21	CS RIGHT SH. BOX
21	2.17	AVOID TO LEFT SH. BOX
40	22.83	CS LEFT SH. BOX
27	5.35	ESCAPE TO RIGHT SH. BOX
41	34.52	CS RIGHT SH. BOX
21	1.85	AVOID TO LEFT SH. BOX
40	48.15	CS LEFT SH. BOX
26	1.49	AVOID TO RIGHT SH. BOX
41	28.51	CS RIGHT SH. BOX
51	0.19	END OF 10-TRIAL BLOCK
22	5.72	ESCAPE TO LEFT SH. BOX
40	29.28	CS LEFT SH. BOX
27	5.39	ESCAPE TO RIGHT SH. BOX
41	34.61	CS RIGHT SH. BOX
22	6.19	ESCAPE TO LEFT SH. BOX
40	23.81	CS LEFT SH. BOX
26	2.85	AVOID TO RIGHT SH. BOX
41	22.15	CS RIGHT SH. BOX
22	6.69	ESCAPE TO LEFT SH. BOX
40	38.31	CS LEFT SH. BOX
26	4.65	AVOID TO RIGHT SH. BOX
41	50.35	CS RIGHT SH. BOX
21	4.62	AVOID TO LEFT SH. BOX
40	45.38	CS LEFT SH. BOX
26	3.40	AVOID TO RIGHT SH. BOX
41	46.48	CS RIGHT SH. BOX
22	6.06	ESCAPE TO LEFT SH. BOX
40	18.94	CS LEFT SH. BOX
26	4.60	AVOID TO RIGHT SH. BOX
41	30.40	CS RIGHT SH. BOX
21	2.96	AVOID TO LEFT SH. BOX

--APPENDIX C--

Sample condensed summary of the data set just presented:

```
*****
SUBJECT NO. :      3                      DATE:  8/20/81
EXP. NO.      GROUP NO.  STA NO.  PROG. NO.  NO. COUNTERS
  0              1          6         7         22
FILE NAME     S00082081.03
*****
```

SESSION START TIME 9: 6

TOTAL RUNTIME OF SESSION 44 MIN. 22.46 SEC.

CODE	FREQ.	SUM	MEAN	LABEL
20 -->	34	260.10	7.65	ENTER LEFT SH. BOX
21 -->	9	28.71	3.19	AVOID TO LEFT SH. BOX
22 -->	22	57.17	2.60	ESCAPE TO LEFT SH. BOX
25 -->	36	235.19	6.53	ENTER RIGHT SH. BOX
26 -->	16	49.51	3.09	AVOID TO RIGHT SH. BOX
27 -->	13	32.73	2.52	ESCAPE TO RIGHT SH. BOX
30 -->	13	65.00	5.00	SHOCK LEFT SH. BOX
35 -->	22	110.00	5.00	SHOCK RIGHT SH. BOX
40 -->	29	859.13	29.63	CS LEFT SH. BOX
41 -->	31	955.42	30.82	CS RIGHT SH. BOX
51 -->	5	19		END OF 10-TRIAL BLOCK
52 -->	5	30		SHAM SUPPRESSION TRIAL
62 -->	1	906		STATION START
63 -->	1	950		STATION STOP

Sample output of latency data extracted from the data set presented and summarized above.

```
*****
SUBJECT NO. :      3                      DATE:  8/20/81
EXP. NO.      GROUP NO.  STA NO.  PROG. NO.  NO. COUNTERS
  0              1          6         7         22
FILE NAME     S00082081.03
*****
```

FOR 10-TRIAL BLOCKS			
BLOCK	MEAN	SD	MEDIAN
1	11.098	4.712	9.583
2	5.500	1.449	5.705
3	4.944	2.107	5.880
4	4.673	1.918	5.065
5	3.356	1.734	2.480
6	4.741	1.360	4.635

FOR 60-TRIAL SESSION

MEAN	SD	MEDIAN	SIQR	FILENAME
5.72	3.463	5.70	1.476	S00082081.03

APPENDIX D: INPUT AND OUTPUT (I/O) LOOK-UP TABLE FOR ESCAPE AND AVOIDANCE TESTING SYSTEM

/SHUTTLE ID -- LISTING OF INPUT/OUTPUT LOOKUP TABLE FOR USE
 /WITH RAT SHUTTLE ESCAPE-AVOIDANCE PROGRAMS. LISTINGS FOR
 /STATIONS 1-3 (MONKEY STATIONS) OMITTED. DEVICE CODES REFER
 /TO 8-BIT BLOCKS OF DIGITAL INPUT OR 16-BIT BLOCKS OF DIGITAL
 /OUTPUT ON THE MANX DIGITAL I/O CARDS.

NO. OF UNIQUE DEVICE CODES 8

1 = 32
 2 = 33
 3 = 34
 4 = 35
 5 = 36
 6 = 37
 7 = 38
 8 = 39

NO. OF INPUT CARDS 8

1 = 32
 3 = 34
 4 = 35
 5 = 36
 6 = 37
 7 = 38
 8 = 39

/INPUTS 1 - 8
 / " 33-40
 / " 49-56
 / " 65-72
 / " 81-88
 / " 97-104
 / " 113-120

NO. OF OUTPUT CARDS 8

1 = 32
 2 = 33
 3 = 34
 4 = 35
 5 = 36
 6 = 37
 7 = 38
 8 = 39

/OUTPUTS 1- 16
 / " 17- 32
 / " 33- 48
 / " 49- 64
 / " 65- 80
 / " 81- 96
 / " 97-112
 / " 113-128

NO. OF STATIONS 6

STATION NO. 4

NO. OF INPUTS 3

1 = 78
 2 = 77
 3 = 71

/LEFT FLOOR SWITCH
 /RIGHT FLOOR SWITCH
 /HAND START SWITCH

NO. OF OUTPUTS 8

1 = 86
 2 = 85
 3 = 94
 4 = 93
 5 = 78
 6 = 77

/LEFT CS LIGHT
 /RIGHT CS LIGHT
 /LEFT HOUSE LIGHT
 /RIGHT HOUSE LIGHT
 /LEFT SHOCKER
 /RIGHT SHOCKER

STATION NO. 5

NO. OF INPUTS 3

1 = 76
 2 = 75
 3 = 70

/INPUTS AND OUTPUTS FOR
 /STATIONS 5 & 6 CORRESPOND
 /TO THOSE FOR STATION 4

--APPENDIX D--

NO. OF OUTPUTS	8
1 = 84	
2 = 83	
3 = 92	
4 = 91	
5 = 76	
6 = 75	

STATION NO.	6
NO. OF INPUTS	3
1 = 74	
2 = 73	
3 = 69	

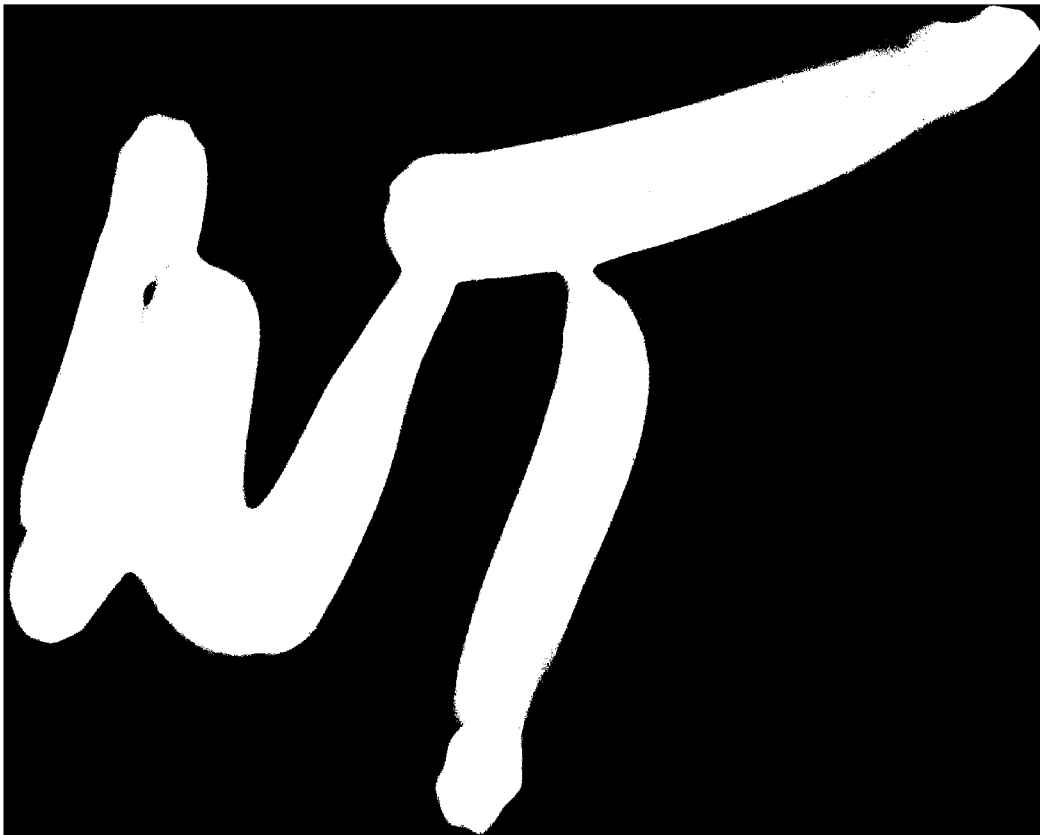
NO. OF OUTPUTS	8
1 = 82	
2 = 81	
3 = 90	
4 = 89	
5 = 74	
6 = 73	

END

~~FILED~~

2-83

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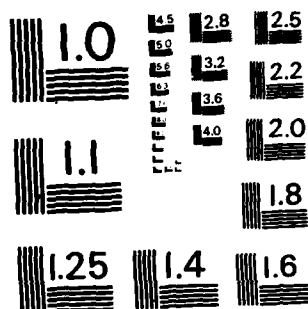
BEHAVIORAL AND PHYSIOLOGICAL EFFECTS OF PSYCHOLOGICAL
STRESS IN RATS(U) SYSTEMS RESEARCH LABS INC DAYTON OH
J LANUM ET AL. NOV 82 SAM-TR-82-34 F33815-80-C-0803
F/G 6/3

22

UNCLASSIFIED

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	SUPPLEMENTARY INFORMATION		END F33815-80-C-0803 F/G 6/3
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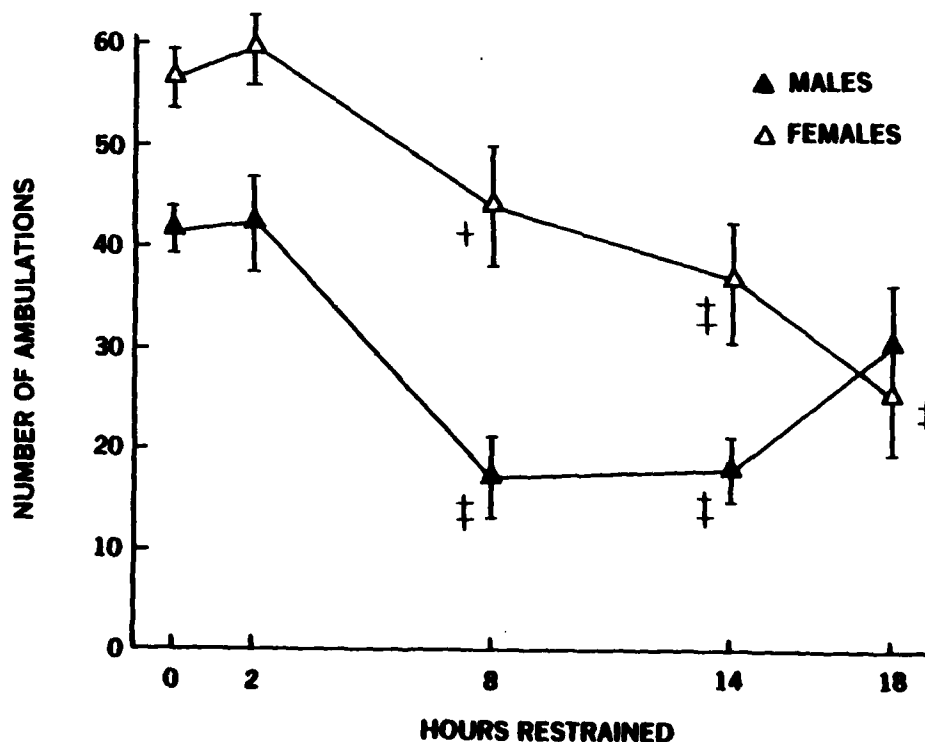
MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS - 1963 - A

SUPPLEMENTARY

INFORMATION

AD-A123428

Errata for: Lanum, J., Campbell, M. E., Blick, D. W., Wheeler, T. G., and Yates, J. T. Behavioral and physiological effects of psychological stress in rats. USAF School of Aerospace Medicine. SAM-TR-82-34, 1982.



- 1) The figure printed on page 12 of the report (Figure 2) should be replaced with this figure.
- 2) The "S.D."s on pp.1&2 and in captions for Figures 1-5, 7, 8, 11, 12, and 14 should be replaced by "S.E.M.."

END

DATE
FILMED

6 83

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