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EFFECTS OF STAPHYLOCOCCAL ENTEROTOXIN B ON COMPLEX OPERANT BEHAVIOR IN MONKEYS

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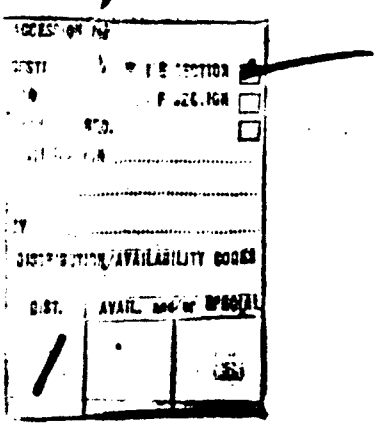
Animals used in this study were handled in accordance with the "Guide for Laboratory Animal Facilities and Care" prepared by the National Academy of Sciences- National Research Council and in accordance with the Secretary of Agriculture Standards in "Laboratory Animal Welfare."



FOREWORD

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This technical report has been reviewed and is approved.

RALPH F. ZIEGLER, LtCol, USAF, VC
Commander

ABSTRACT

Four female rhesus macaques (Macaca mulatta), weighing 4.31 to 5.45 kg., demonstrated significant performance decrements following gastric infusion of 20 micrograms per kilogram of staphylococcal enterotoxin B on shock avoidance schedules (CA and DA) judged high activity, high concentration, and on positive reinforcement schedules (VISAC) and (DRL) judged medium activity, high concentration, and low activity, medium concentration, respectively. Results, obtained under a controlled atmosphere of 10,000 feet simulated altitude and 100 percent oxygen, indicated individual toxicological effects on behavior with avoidance schedules showing a stress level difference.

INTRODUCTION

Subhuman primate behavioral changes, attributed to toxicity of various drugs or compounds, occupy a small portion of existing toxicological and pharmacological literature (1). The majority of toxicological investigations deal with physiological effects from which behavioral changes are inferred (2). It is, however, of qualitative interest to determine the degree of impairment exposure to toxic compounds may have on an organism's ability to function within established baseline standards.

The purpose of this study was to explore toxicological effects of staphylococcal enterotoxin B, a compound frequently associated with food poisoning, on operant conditioning schedules of reinforcement assigned to specific stress conditions among elements of a performance flight profile.

METHOD

A. Subjects

Four female rhesus macaques (Macaca mulatta), ranging in weight from 4.31 kg. to 5.45 kg. on the first day of experimentation, served as the subjects of this study. All macaques were maintained on 90 percent normal body weight food deprivation schedules, supplemented with vitamins. Handling and seating operations were in accordance with procedures developed and currently used in the laboratory (3).

B. Apparatus

1. Programming Equipment

Programming equipment consisted of solid state modules which provided schedules for training and experimental contingencies. Digital counters were used to record all correct and incorrect responses, number of lever responses, and total reinforcements delivered. Digital printouts recorded reaction times (RT) within .01 second. Cumulative recorders were used to record continuous avoidance (CA) response rates and correct visual acuity (VISAC) and differential reinforcement at low rates (DRL) responses (Fig. 1).

2. Performance Panel

The four aluminum performance panels were 26.0 cm. wide by 44.5 cm. high. The panels were mounted adjacent to each other and were constructed on a single base which also served to support the four primate chairs (Fig. 2).

Located on each panel were three 5 cm. x 5 cm. stimulus-response keys (SRK), two 2.5 cm. diameter stimulus lights, two response levers, one miniature rear projection inline

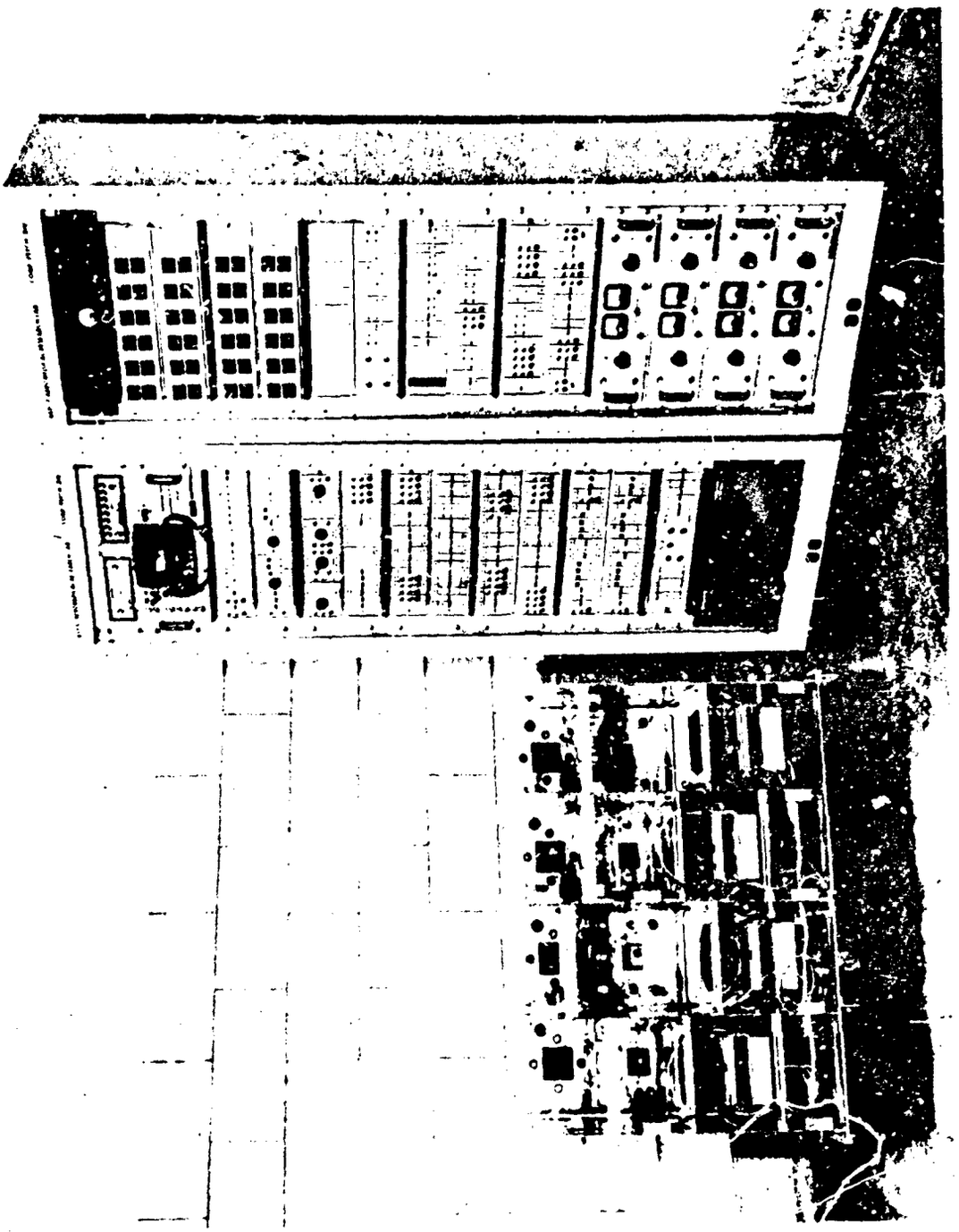


Figure 1. Programming Equipment

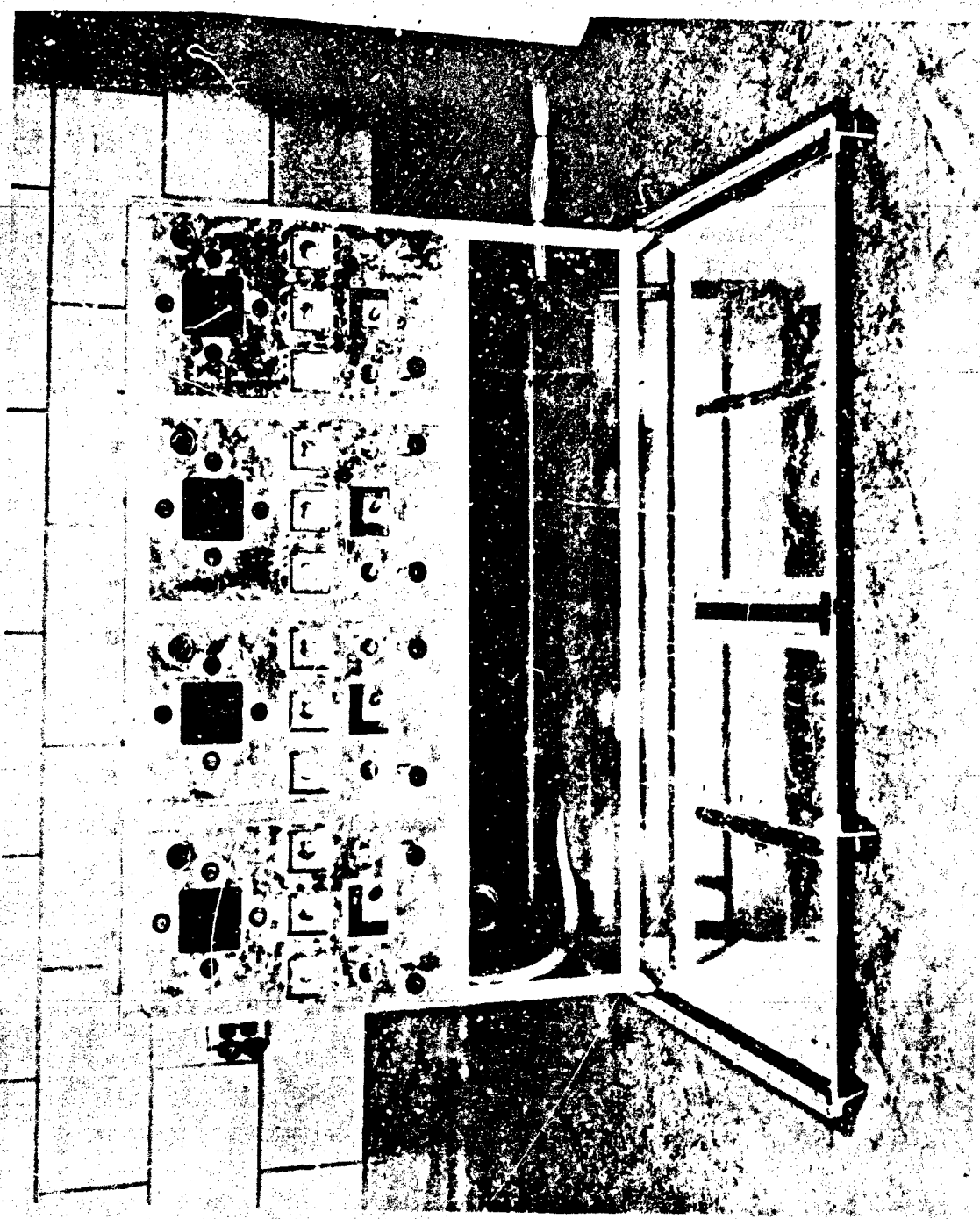


Figure 2. Performance Panel

digital display (IDD), four response levers positioned around the IDD readout assembly, one food pellet hopper, and one external loudspeaker baffle (Fig. 3).

3. Primate Restraint Unit

Four aluminum primate restraint chairs were constructed and mounted adjacent to each other to form a single assembly. Each chair contained an adjustable waist and neck plate to allow positioning of the subjects in relation to the performance panel. Four independent training and testing situations were established when the primate restraint chair assembly, with the seated primates, was connected to the panel and base assembly.

Shock stimulation was provided by four 1.3 cm. stainless steel rods which served as the seat in each chair (Fig. 4).

During avoidance, current was passed from the four positive seat rods to an indifferent ground electrode attached to the leg of each macaque.

4. Toxin Dosage and Administration

In a pilot study prior to the experimental drug administration an effective dose level was determined at 20 micrograms of staphylococcal enterotoxin B per kilogram body weight for the rhesus. The toxin was mixed with 10 ml. for the experimental administration via nasal gastric tube, and then flushed with an additional 3 ml. saline. Sham administrations were accomplished just prior to the "take-off" flight element.

5. Environmental Altitude Chamber

An altitude chamber, located at the USAF School of Aerospace Medicine, Brooks Air Force Base, Texas, provided the control environment during stabilization training, baseline and experimental testing. Among the variables controlled in this environment were altitude, percent oxygen density, temperature, and humidity (4).

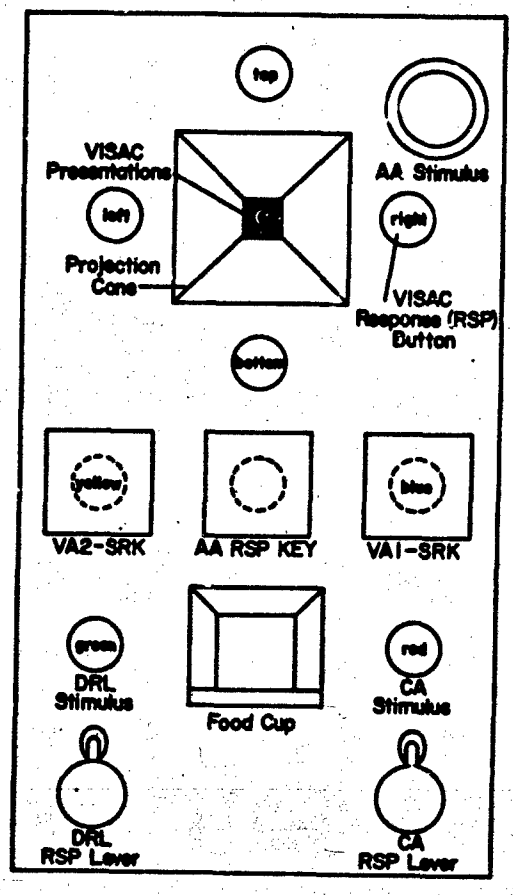


Figure 3. Schematic Performance Panel

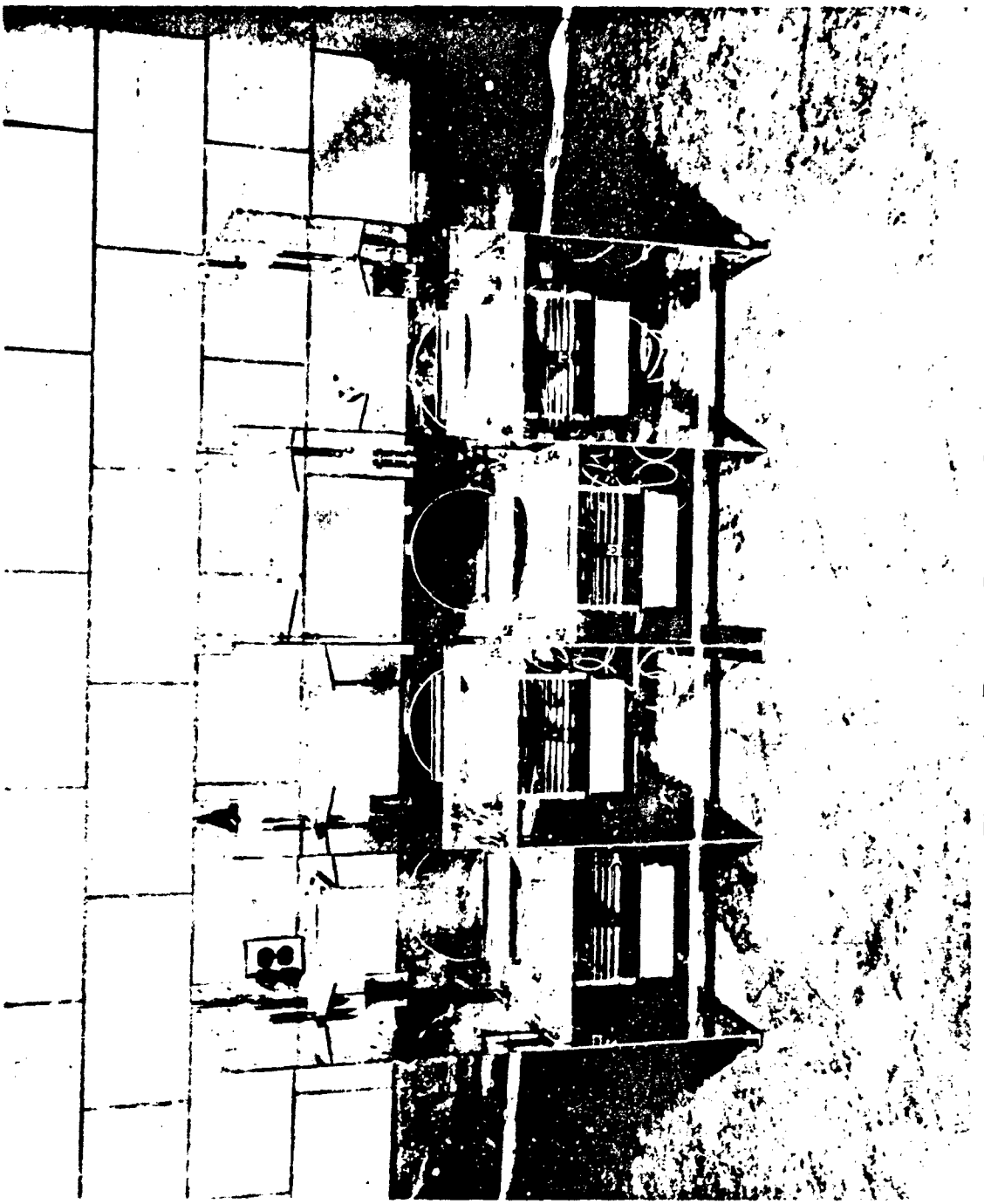


Figure 4. Primate Restraint Unit

C. Performance Schedules

Performance schedules were selected and arranged among elements of the flight profile on the basis of hypothesized stress level activity and concentration, and capacity to measure change in basic performance operations necessary for the successful accomplishment of higher-order complex flight operations encountered in each element heading. Stress level conditions as well as other pertinent flight profile conditions are presented in Table I.

Two types of performance schedules were employed in this study: (1) avoidance or negative reinforced schedules, and (2) positive reinforced schedules.

1. Avoidance Module

Avoidance consisted of forty-three 4-minute CA sessions, with 31 CA sessions accompanied by discrete avoidance (DA) presentations. The CA task had a response-shock (RS) interval of 5 seconds and required continuous lever monitoring during 4-minute sessions.

Subjects were required to respond at least once every 5 seconds on the CA response lever to avoid negative reinforcement at termination of the RS interval. The discriminative stimulus was a red light in the lower right-hand corner of the performance panel positioned just above the CA response lever.

Two types of DA tasks, visual avoidance (VA) and auditory avoidance (AA), (5) accompanied 31 CA task presentations (CA and DA) except during 12 sessions along the flight profile where the CA task was the only task presented (Fig. 5). During CA and DA sessions, the temporal sequence of DA presentations remained constant although unequally spaced within this sequence.

DA tasks were signaled by either light cues, appearing within the SRK's for the VA tasks -- VA1 blue and VA2 yellow,

TABLE I. FLIGHT PROFILE CONDITIONS

FLIGHT SEQUENCE		STRESS LEVEL		SIMULATED TASK OPERATIONS AND EXPERIMENTAL ENVIRONMENT	
SIMULATED ELEMENTS	DURATION	ACTIVITY	CONCENTRATION	PROGRAM PRESENTED	CHAMBER CONDITIONS
1. PREFLIGHT	1 HR.	LOW	LOW	PURE CA (3)* DRL (2)	AMBIENT
2. TAKE-OFF and CLIMB	30 MIN.	HIGH	HIGH	CA and DA (6)	CLIMB AT 100% O_2
3. CRUISE	2 HR.	LOW	MEDIUM	VISAC (4) DRL (4) CA (4)	STABILIZE ALTITUDE AT 100% O_2
4. REFUEL	30 MIN.	MEDIUM	HIGH	VISAC (1) DRL (1) CA and DA (1)	HUMIDITY - 35% TEMP. - 70°F
5. CRUISE	30 MIN.	LOW	MEDIUM	VISAC (1) DRL (1) CA (1)	SAME
6. LOW LEVEL RUN	2 HR.	HIGH	HIGH	CA and DA (17)	SAME
7. REFUEL	30 MIN.	MEDIUM	HIGH	VISAC (1) DRL (1) CA and DA (1)	SAME
8. CRUISE	2 HR.	LOW	MEDIUM	VISAC (4) DRL (4) CA (4)	SAME
9. LAND	30 MIN.	HIGH	HIGH	CA and DA (6)	DESCENT TO AMBIENT

*NUMBER OF CONTINGENT TASK REPLICATIONS

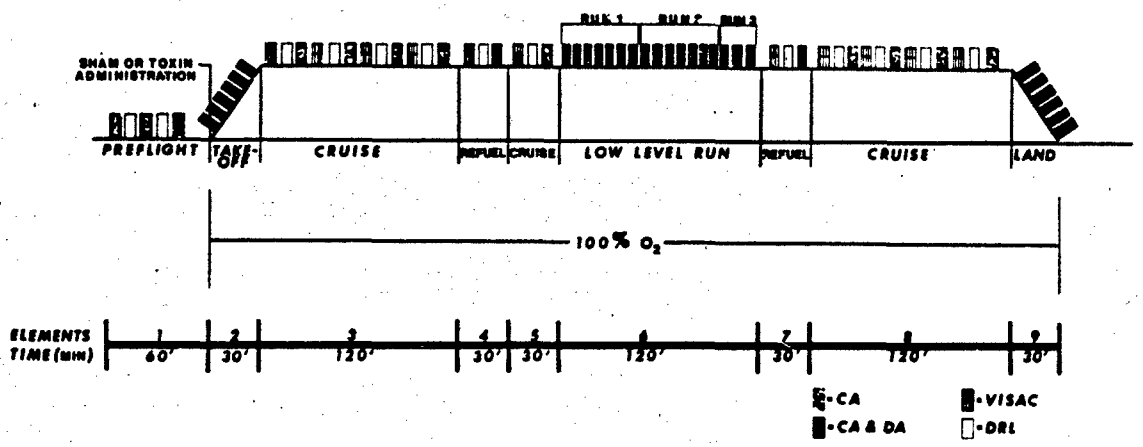


Figure 5. Schematic Test Profile

or by an auditory signal -- 1000 hzs presented by a miniature loudspeaker for the AA task. DA task cues had presentation intervals of 3 seconds. Failure of a subject to respond to the appropriate SRK (Fig. 2) during presentation of a VA1, VA2, or AA cue (DA cues never overlapped) resulted in negative reinforcement at termination of the cue.

2. Positive Reinforcement Module

Two types of positive reward tasks were employed: (1) VISAC and (2) DRL.

The VISAC task consisted of 24 Landolt ring problems (6) which were randomly distributed among 24 fixed trial positions during each 8 minute VISAC session (Fig. 5). The problems were constructed from ring sizes of visual angles corresponding to visual acuities of 20/100, 20/200, and 20/400. During 10-second trial presentations, a subject had to respond to the response key opposite the gap in a presented Landolt ring (Fig. 2) to obtain food pellet reward. Following termination of a trial, or any response made during a trial, was a 10-second non-contingent period before presentation of the next trial in the sequence.

The final positive rewarded task was a 15-second DRL schedule (7), which served as a measure of temporal discrimination. Contingencies required the subjects to discriminate time by spacing responses at least 15 seconds apart in order to receive a food pellet reinforcement. This was presented for 5 minutes during which time subjects responded in the presence of a green discriminative stimulus light located above the DRL lever (Fig. 2).

D. Procedure

The four subjects received extensive training utilizing operant techniques, and were required to demonstrate asymptotic levels of performance on all behavioral tasks prior to on-site

testing. The subjects were then transferred to the chamber environment, and were observed over a period of 3 days to maintain asymptotic levels of performance across all elements of the flight profile (Fig. 5).

Baseline data were obtained from the last stabilization session in which no unusual variations in performance were observed. During on-site training and testing conditions, all subjects were required to perform 5 hours and 25 minutes every other day in an altered atmosphere of 100 percent oxygen (O_2) at a simulated altitude of 10,000 feet. A total of seventeen 12-minute rest periods were provided during each 9.5 hour performance training or testing day and followed pre-flight CA sessions, the take-off element, cruise CA sessions, refuel CA and DA sessions, and two CA and DA 7-sessions and one 3-session low-level-run. Also, during this time, twenty-eight 1-minute and twenty-four 30-second rest periods occurred. One-minute and 30-second rest periods followed each CA and DA presentation and positive reward task, respectively, except where 12-minute rest periods were scheduled. One additional minute was utilized in countdown operations.

At completion of the pre-flight performance program, the chamber door was opened, and the performance module (Fig. 5) was placed outside the chamber and the control or experimental variable was administered. The performance module was then returned to the chamber and performance conditions were resumed.

All training and baseline testing sessions were accompanied with a sham administration of saline delivered into the stomach of each macaque, via gastric tube, at completion of the pre-flight performance sessions. During experimental sessions, 3 ml. saline was used to flush the toxin through the gastric tube.

E. Statistical Samples and Tests

1. Avoidance Data

Data obtained under conditions of the CA and DA tasks were analyzed differently. CA data points consisted of total CA responses for each 4-minute performance session with "n" representing the number of occurrences of 4-minute CA performance sessions in any designated flight proflie element. Data occurring under DA task conditions were analyzed in terms of RT's. Four presentations of each of the three DA tasks (VA1, VA2, and AA) resulted in four RT's per task for each avoidance session. Mean RT's were then computed for each task for each avoidance session by dividing the sum of the four RT's by four. This gave a single mean RT value for each DA task for each session with an "n" of one. Total DA for each session had an "n" of three and was the sum of the three RT means. Therefore, if six avoidance sessions occurred in a specific element of the flight profile, there would be six data points for each DA task and 18 data points for total DA.

Baseline and experimental comparisons of avoidance behavior were made for each element and low level run segment of the flight profile for each subject and for combined subjects. Significant avoidance decrements beyond a .05 confidence limit were determined with a "t" (one-tail) test of significance for uncorrelated means.

2. Positive Reward Data

Response data occurring under conditions of the VISAC and DRL tasks were analyzed in terms of percent efficiencies. A data point was determined for each VISAC session of 24 problems by dividing total correct responses by total number of VISAC presentations multiplied by 100. A data point for each DRL session was calculated by dividing total correct responses by total number of possible correct responses

multiplied by 100. Total possibilities were computed by dividing the total DRL session time of 300 seconds by the DRL interval of 15 seconds.

Medians and semi-interquartile ranges were computed to make visual comparisons of baseline and experimental combined subjects. A Wilcoxon Matched-Pairs Signed-Ranks Test (8) (T one-tail) was utilized to test for significant changes in magnitude and direction of difference of percent efficiency data beyond a .05 confidence limit.

RESULTS

A. Avoidance Schedules

In Table II are presented significant performance decrements from baseline and experimental "t" comparisons of uncorrelated avoidance means during each element and low level run segment of the flight profile for each subject.

Significant performance decrements from baseline and experimental "t" comparisons of uncorrelated avoidance means and "t" comparisons of percent efficiencies during each element and low level run segment of the flight profile for combined subjects are presented in Table III.

B. Positive Reward Schedules

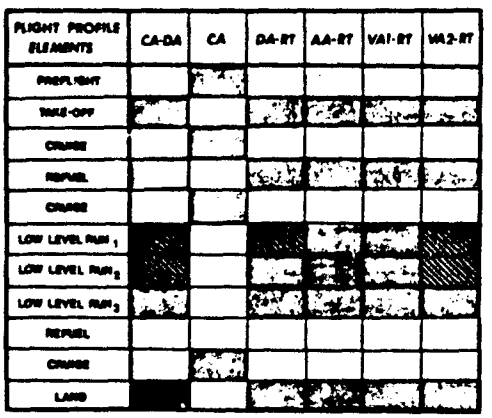
In Table IV are presented medians and semi-interquartile ranges computed from baseline and experimental percent efficiency data for each element of the flight profile for combined subjects.

C. Environmental Controls

Baseline and experimental chamber atmospheric alterations from ambient were introduced with the beginning of the take-off

TABLE II. SIGNIFICANT AVOIDANCE DECREMENTS FOLLOWING SEB ADMINISTRATION

SUBJECT 1



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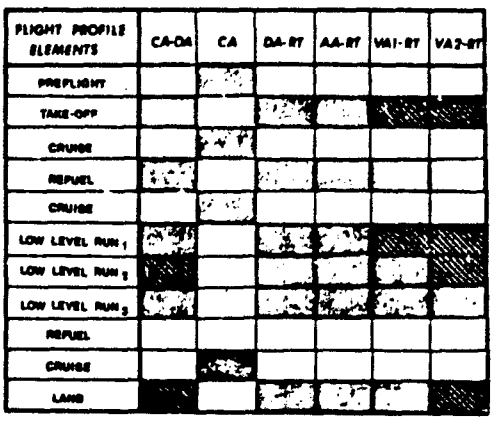

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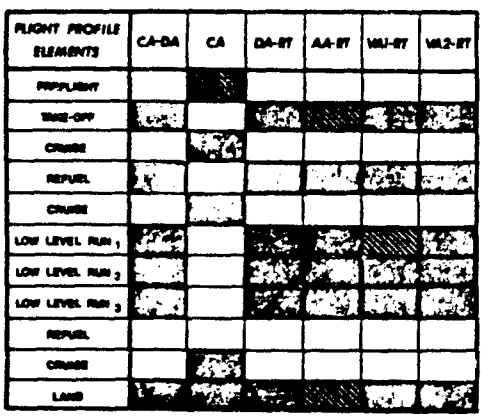

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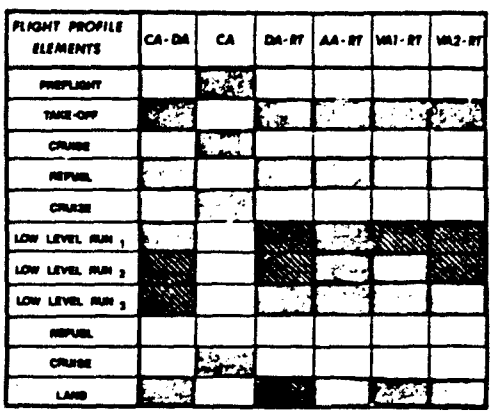

SIGNIFICANT DECREMENT BEYOND .05 CONFIDENCE LEVEL FOR "t" (ONE TAIL)

NOT SIGNIFICANT

NO PRESENTATION

TABLE III. SIGNIFICANT PERFORMANCE DECREMENTS FOLLOWING SEB ADMINISTRATION FOR COMBINED SUBJECTS

FLIGHT PROFILE ELEMENTS	CA-DA	CA	DA-RT	AA-RT	VA1-RT	VA2-RT	VISAC	DRL
PREFLIGHT								
TAKE-OFF								
CRUISE								
REFUEL							— —	
CRUISE								
LOW LEVEL RUN 1								
LOW LEVEL RUN 2								
LOW LEVEL RUN 3								
REFUEL								
CRUISE								
LAND								

SIGNIFICANT DECREMENT BEYOND .05 CONFIDENCE LEVEL FOR "t" AND WILCOXON MATCHED-PAIRS SIGNED-RANKS TEST "T" (ONE TAIL)

NOT SIGNIFICANT

NO PRESENTATION

— — DATA INSUFFICIENT FOR ANALYSIS

TABLE IV. POSITIVE REINFORCEMENT TASK EFFICIENCIES

VISUAL ACUITY PERCENT EFFICIENCY						
FLIGHT PROFILE ELEMENTS	BASELINE			EXPERIMENTAL		
	N*	MDN**	Q***	N*	MDN**	Q***
3. CRUISE	16	92.17%	4.00	16	88.50%	48.00
4. REFUEL	4	96.50%	2.00	3	00.50%	21.25
5. CRUISE	4	96.00%	4.00	4	00.17%	00.33
7. REFUEL	4	96.00%	2.00	4	00.17%	00.33
8. CRUISE	16	96.10%	3.92	16	00.50%	06.25
DRL PERCENT EFFICIENCY						
FLIGHT PROFILE ELEMENTS	BASELINE			EXPERIMENTAL		
	N*	MDN**	Q***	N*	MDN**	Q***
1. PREFLIGHT	8	65.00%	2.50	8	70.50%	06.09
3. CRUISE	16	55.50%	10.00	16	60.50%	35.10
4. REFUEL	4	62.50%	7.50	4	00.17%	00.33
5. CRUISE	4	72.50%	10.00	4	00.17%	00.33
7. REFUEL	4	65.00%	5.00	4	00.17%	00.33
8. CRUISE	16	65.25%	7.38	16	00.23%	05.32

*SUM OF CLASS-INTERVAL FREQUENCY
**MEDIAN
***SEMI-INTERQUARTILE RANGE

element, and reached a plateau after 30 minutes from onset. Oxygen concentration at plateau was 100 percent and altitude was maintained at approximately 10,000 feet (522 mm. Hg.). Temperature and humidity ranged from 65°F to 75°F and 20 percent to 50 percent, respectively.

D. Summary of Performance Decrements

1. Element 1 -- Pre-flight

For combined subjects, no significant performance decrements occurred during 12 sessions of CA and eight sessions of DRL. Median DRL efficiency increased by 5.50 percent with greater variability in the experimental sample (Q = 2.50 to Q = 5.09).

For individual subjects, a significant decrement occurred with subject No. 3 during three sessions of CA.

2. Element 2 -- Take-off and Climb

During 24 CA and DA sessions for combined subjects, a significant performance decrement occurred in RT on the VA2 task.

For individual subjects, significant performance decrements occurred in RT during six sessions each of VA1 and VA2 for subject No. 2 and on AA for subject No. 3.

3. Element 3 -- Cruise

No significant decrements occurred during 16 sessions of CA, VISAC, and DRL for combined subjects. Median VISAC efficiency decreased by 3.67 percent with greater variability occurring in the experimental sample (Q = 4 to Q = 48). Median DRL efficiency increased by 5 percent with greater variability occurring in the experimental sample (Q = 10 to Q = 35.10).

No significant decrement occurred during four sessions of CA for individual subjects.

4. Element 4 -- Refuel

During four CA and DA, VISAC, and DRL sessions for combined subjects, a significant decrement occurred with the DRL task. A decrement was observed in VISAC efficiency but due to the small sample "n", a significant comparison was not possible. Median VISAC efficiency decreased by 96 percent with greater variability occurring in the experimental sample (Q = 2.00 to Q = 21.25). Median DRL efficiency decreased by 62.33 percent with greater variability occurring in the baseline sample (Q = 7.50 to Q = 21.25). There was greater baseline sample variability since three of the experimental sample scores were zero. It is interesting that subject No. 1 showed no change in efficiency on VISAC (100 percent) and an increase by 5 percent on DRL while extreme decrements were observed for all other subjects on these tasks (not shown).

For one session each of CA and DA for individual subjects, a test of significance was not possible. However, all subjects except subject No. 4 exhibited a decrement on the CA task, while on DA tasks, performance decrements occurred on VA1 for subject No. 4; on VA2 for subjects No. 2, No. 3, and No. 4; and on AA for subject No. 3.

5. Element 5 -- Cruise

For combined subjects on four sessions of CA, VISAC, and DRL a significant decrement occurred on VISAC and DRL tasks. Median VISAC efficiency was decreased by 95.83 percent with greater variability in the baseline sample (Q = 4.00 to Q = 0.33). Median DRL efficiency was decreased by 72.33 percent with greater variability in the baseline sample (Q = 10.00 to Q = 0.33). It should be noted that subject No. 1 showed an increase by 12 percent on VISAC efficiency and a decrease of only 15 percent on DRL efficiency, while the other subjects exhibited extreme decrements to zero efficiency on these tasks.

During one session of CA, decrements occurred for mean lever responses for all subjects except subject No. 4.

6. Element 6 -- Low Level Run

a. Low Level Run I

During 28 sessions of CA and DA for combined subjects, a significant decrement occurred in RT on the DA, VA1, and VA2 tasks.

For individual subjects, significant decrements in RT occurred during seven sessions of CA and DA on CA, DA, and VA2 for subject No. 1; VA1 and VA2 for subject No. 2; VA1 for subject No. 3; and DA, VA1, and VA2 for subject No. 4.

b. Low Level Run II

During 28 sessions of CA and DA for combined subjects, a significant decrement occurred in RT on the DA and VA2 tasks.

For individual subjects, significant decrements occurred during seven sessions of CA and DA on CA, and VA2 for subjects No. 1 and No. 2; and on CA, DA, and VA2 for subject No. 4.

c. Low Level Run III

During 12 sessions of CA and DA for combined subjects, no significant performance decrements occurred.

For individual subjects, a significant decrement in mean CA lever responses occurred for subject No. 4 during three sessions of the CA and DA task.

7. Element 7 -- Refuel

During four CA and DA, VISAC, and DRL sessions for combined subjects, significant efficiency decrements occurred on the VISAC and DRL tasks. Median VISAC efficiency data decreased by 95.83 percent with greater variability in the baseline sample. It is interesting to note that subject No. 1 decreased in VISAC efficiency by 25 percent and in DRL efficiency by 50 percent while all other subjects exhibited extreme decrements to zero on these tasks.

For one session each of CA and DA, for individual subjects, all subjects except subject No. 2 showed a decrease in mean CA lever responses. On DA tasks, performance decrements in RT occurred on total DA for all subjects except subject No. 2, on VA1 for subjects No. 3 and No. 4, on VA2 for all subjects except subject No. 4. Because of the small "n" a test of significance was not possible.

8. Element 8 -- Cruise

During 16 sessions of CA, VISAC, and DRL, significant decrements occurred on the VISAC and DRL tasks. Median VISAC efficiency data decreased by 95.50 percent with greater variability in the experimental sample ($Q = 3.92$ to $Q = 6.25$). Median DRL efficiency data decreased by 65.02 percent with greater variability in the baseline sample ($Q = 7.38$ to $Q = 5.32$). Although all subjects showed extreme decrements on the VISAC and DRL tasks, subject No. 1 showed the least decrement with subject No. 4 next in line.

No significant decrements occurred on CA during four sessions for individual subjects.

9. Element 9 -- Land

No significant performance decrements occurred during 24 sessions of CA and DA for combined subjects.

For six sessions of CA and DA for individual subjects, significant performance decrements occurred on CA for subject No. 1, CA and VA2 for subject No. 2, AA for subject No. 3, and DA for subject No. 4.

DISCUSSION

In order to prepare a behavioral program sensitive to toxicological effects following SEB administration, performance tasks were selected and combined to provide sensory and motor functions common to the most primitive primate species. Although elementary, these component aspects of behavior form the basis of complex operations governing higher intellectual functions, such as the operations of a complex vehicle.

Avoidance tasks were differentially impaired for individual subjects following SEB administration. A greater number of extreme subject differences occurred among more of the subjects under conditions of shock than with conditions of positive reinforcement.

For individual subjects, the avoidance module (CA and DA), occurring with elements judged high activity, high concentration, showed the majority of significant avoidance decrements (see Tables I, II, III). It can therefore be assumed that the avoidance module was an effective indicator of toxicological effect under high stress conditions for the combined sample.

During Low Level Runs II and II, successive sample improvement is suggested which does not appear to be specifically practice effect as no decrement is seen under the same conditions 2 1/2 hours later in element 8.

Positive reward tasks were also differentially impaired following SEB administration, however, subject No. 1 showed the least impairment in efficiency throughout the test. During elements 7 and 8, subject No. 1 showed successively larger

decrements in efficiency. Subjects No. 3 and No. 4 showed slight improvements in VISAC efficiency and subject No. 4 in DRL efficiency during element 8.

In terms of relative stress levels, no performance decrement was observed for VISAC and DRL task during elements 1 and 3, judged low activity, medium concentration, respectively. However, elements 5 and 8 also judged low activity, medium concentration, showed significant decrements for combined subjects with a majority of decrements for individual subjects during the same task configuration as element 3 (Table I). Since all other elements containing VISAC and DRL tasks showed significant decrements for individual subjects, it is impossible to conclude stress level differences.

Results from positive reward schedules suggest differential subject resistance to SEB with some indications of recovery in subjects No. 3 and No. 4 during element 8, and increasing decrement in subject No. 1 during elements 7 and 8. In this regard, the less demanding positive reward schedules may be effective indicators of subtle toxicological effect which might otherwise be initially overlooked by the more demanding emergency requirements of avoidance schedules.

SUMMARY AND CONCLUSIONS

The present study was conducted to investigate differential effects of SEB on complex operant behavior.

Shock avoidance schedules, involving visual and auditory reaction times (DA), combined with a continuous motor requirement (CA), were shown to be sensitive indicators of stress conditions judged high activity, high concentration.

Positive reinforcement schedules, involving visual and matching (VISAC) and temporal pacing of responding (DRL)

were shown to be equally sensitive to stress conditions judged medium activity, high concentration, and low activity, medium concentration.

A greater number of extreme subject differences occurred more often under conditions of avoidance than with conditions of positive reinforcement.

Results indicate individual toxicological effects on behavior with shock avoidance schedules sensitive to high stress conditions. Positive reward schedules are suggested to be sensitive to subtle toxicological effects involving free choice appetitive behavior.

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13. ABSTRACT

Four female rhesus macaques (Macaca mulatta), weighing 4.31 to 5.45 kg., demonstrated significant performance decrements following gastric infusion of 20 micrograms per kilogram of staphylococcal enterotixin B on shock avoidance schedules (CA and DA) judged high activity, high concentration, and on positive reinforcement schedules (VISAC) and (DRL) judged medium activity, high concentration, and low activity, medium concentration, respectively. Results, obtained under a controlled atmosphere of 10,000 feet simulated altitude and 100 percent oxygen, indicated individual toxicological effects on behavior with avoidance schedules showing a stress level difference.

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