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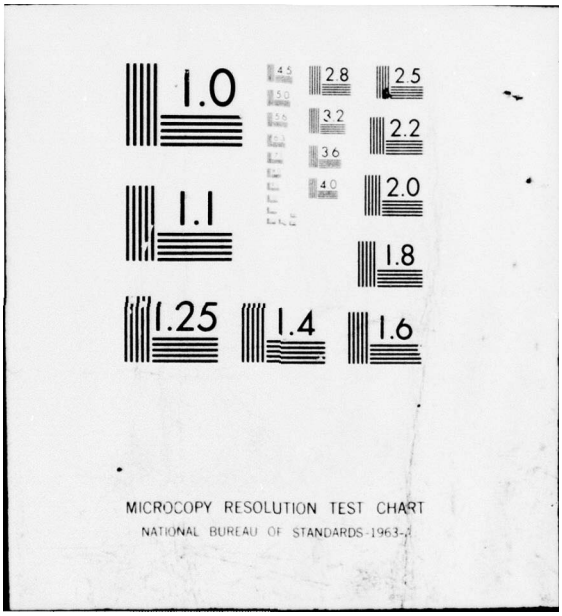
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LAIR REPORT NO. 46

# FIXED RATIO PERFORMANCE IN RATS FOLLOWING MASSIVE TRANSFUSION WITH CELL-FREE RESUSCITATING SOLUTIONS



DEPARTMENT OF BIOMEDICAL STRESS

DEPARTMENT OF SURGERY

JAN 1978



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Rats were trained to perform a fixed ratio (FR) bar press task in order to obtain food reinforcement. FR operant behavior was then examined daily following 65 percent exchange transfusion with bovine serum albumin (BSA), stroma-free hemoglobin (SFH)or a mixtured of those materials. A fourth group of rats (controls) was subjected to identical surgical and anesthetic procedures, but was not transfused. Average FR response rates of all transfused groups were significantly depressed in comparison to surgical controls 24 hr after exchange transfusion. The degree of this initial response depression was inversely (cont)

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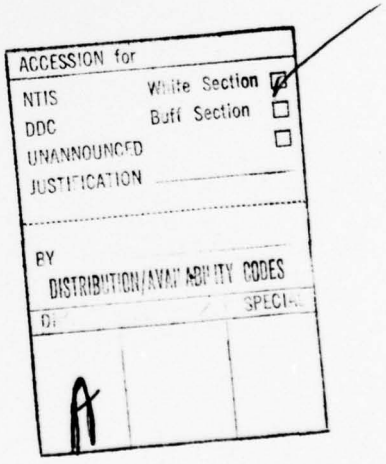
related to baseline FR response rates. Histograms of interresponse times (IRTs) were also derived. Following exchange transfusion, there were increases in the relative proportions of long IRTs and a slowing of IRTs representing fast response sequences. Changes in FR response rates and in IRT distributions also depended upon the type of transfusion solution employed. Rats transfused with MIX were least affected although the differences between this group and the BSA group were not significant. Rats transfused with SFH showed the greatest initial response decrement. The recovery of the SFH group was also significantly longer than those of the control and MIX groups. There were no significant between-group differences in FR performance by the second week of recovery.

## ABSTRACT

Rats were trained to perform a fixed ratio (FR) bar press task in order to obtain food reinforcement. FR operant behavior was then examined daily following 65 percent exchange transfusion with bovine serum albumin (BSA), stroma-free hemoglobin (SFH), or a mixtured of those materials. A fourth group of rats (controls) was subjected to identical surgical and anesthetic procedures, but was not transfused. Average FR response rates of all transfused groups were significantly depressed in comparison to surgical controls 24 hr after exchange transfusion. The degree of this initial response depression was inversely related to baseline FR response rates. Histograms of interresponse times (IRTs) were also derived. Following exchange transfusion, there were increases in the relative proportions of long IRTs and a slowing of IRTs representing fast response sequences. Changes in FR response rates and in IRT distributions also depended upon the type of transfusion solution employed. Rats transfused with MIX were least affected although the differences between this group and the BSA group were not significant. Rats transfused with SFH showed the greatest initial response decrement. The recovery of the SFH group was also significantly longer than those of the control and MIX groups. There were no significant between-group differences in FR performance by the second week of recovery.

## PREFACE

In conducting the research described in this report, the investigator(s) adhered to the Guide for Laboratory Animal Facilities and Care as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care, of the Institute of Laboratory Animal Resources, National Academy of Science - National Research Council.

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# FIXED RATIO PERFORMANCE IN RATS FOLLOWING MASSIVE TRANSFUSION WITH CELL-FREE RESUSCITATING SOLUTIONS

## INTRODUCTION

A fixed ratio (FR) schedule provides reinforcement (RF) for every $n^{th}$ response emitted by the subject. FR performance is easily established in a wide variety of species and produces readily quantified stable patterns of behavior. Two features of FR responding, specifically the temporal characteristics of reinforcement-response and response-response intervals, have been extensively studied. The duration of the period of non-responding between a reinforcement and the first post-reinforcement response has been related to ratio size (1-3), effects of food deprivation (1), drugs (4), brain damage (1), and to the effects of complex schedules (5). In contrast to the highly variable nature of the post-reinforcement pauses, the series of responses which precede reinforcements may occur at a remarkably uniform rate. These episodes of characteristically stable, rapid responding are relatively unaffected by ratio size (2,5), levels of food deprivation (6), delay of reinforcement (7), magnitude of reinforcement (8), and drugs (4).

Previous studies conducted in this laboratory have shown that fixed ratio response rates slowed following massive transfusion with cell-free resuscitating solutions. It was evident when observing subjects that reductions in overall response rates were due to primarily to long periods of non-responding. It was also found that even severely affected subjects were apparently able to sustain responding at moderately high rates over short periods of time. However, it was not possible on the basis of the gross response rate data available from these preliminary studies to determine whether or not such brief periods of sustained responding were comparable to those observed under control conditions. The purposes of the present experiment were to replicate the results of earlier work and to examine critically changes in portions of interval histograms of inter-response times (IRTs) corresponding to maximum FR response rates.

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## METHODS

### Subjects

The subjects were twenty-one male outbred Sprague-Dawley rats weighing an average of 317 g (range 262-368 g) at the time of transfusion. Rats were maintained under constant temperature and humidity and in a reversed 12 hr light-dark cycle, i.e., with the rats' dark cycle corresponding to normal laboratory work hours. Throughout the experiment, subjects were provided water ad libitum while in their home cages. Food deprived rats obtained food as reinforcement during operant testing and were given a supplemental 20-25 g food ration immediately following each session. Additional food rations were provided on weekends to compensate for food not obtained during daily FR sessions. None of the animals used in the present experiment had been used for previous studies.

### Apparatus

Seven modular small animal test cages obtained from Coulbourn Instruments were used in this experiment. Each cage was housed in an isolation cubicle equipped with an exhaust fan which provided both ventilation and background masking noise. Operation of a response lever dispensed 45 mg Noyes food pellets into a trough located next to the lever. The response lever was located 3 cm above the cage floor. Each cage was illuminated with a No. 1819 bulb which remained on throughout the sessions. Operant devices in the seven test cages were independently controlled by solid-state digital logic devices. Binary code pulses uniquely identified response or reinforcement events for each box. These were recorded on FM magnetic tape for subsequent analysis with a PDP-12 computer.

### Training

Preliminary training of rats to lever-press for food required approximately three days. All rats were operating on a continuous reinforcement (one food pellet/response) schedule by the end of the first week of training. During the second week, the ratio of responses per food pellet was changed to 20 and maintained at this level throughout the experiment. Subjects were run for 30 min, 5 days per week during 8 weeks of training and daily for 10 days before and after treatment. Post-treatment sessions were then continued on the 5-day/wk schedule for one month. The ten-day pre- and post-treatment sessions were designated "baseline" and "recovery" periods for purposes of analysis. Following training, the twenty-one subjects were divided into four groups of five rats (with one extra subject) with each group assigned to one of four treatment conditions. Three groups were designated to receive transfusions. The fourth group was exposed to identical surgical and anesthetic procedures but was not transfused.

## Transfusion

Seven percent solutions (in kidney dialysis fluid) of bovine serum albumin (BSA), stroma-free hemoglobin (SFH) or a mixture (MIX) containing 7% SFH and 7% BSA were used as transfusion materials. The formulation of BSA and SFH solutions used have been described previously (9,10). Prior to transfusion, subjects were anesthetized with diethyl ether. The right jugular vein was then exposed and a PE 90 polyethylene catheter was inserted through the vein into the right atrium. The catheter was attached to two syringes via a 3-way, sterile, nonpyrogenic stopcock. One syringe contained donor material and the other served as a reservoir for blood removed from the recipient. Exchange transfusion was then accomplished in 1 ml increments until an approximate 65 percent reduction in hematocrit was observed. The transfusion process required about 15 min to complete. Following transfusion, the catheter was removed and the jugular vein ligated. Subjects were returned to their home cages where rapid recovery from anesthetic effects occurred. All rats were allowed to recover 24 hr and were then returned to the laboratory for routine daily behavioral testing.

## Data Analysis

Response totals were routinely collected from subjects during each experimental session. Baseline response rates were obtained from each subject by averaging the response totals obtained during the 10 consecutive daily sessions preceeding transfusion or surgical control procedures. Daily recovery response totals were converted to "output ratios" by expressing scores as a percentage of each subject's mean baseline response rate. The resulting data were the principle source of information concerning overall effects of the experimental conditions on gross FR response rates. Output ratios or percent change scores facilitate comparison among subjects or groups but do not always compensate for pretreatment differences in response rates. This is due in part to the existence of rate-dependent effects in which the level of baseline responding partially determines the magnitudes of changes in recovery response rates. The occurrences of such effects had been suggested in earlier pilot studies of FR performance and transfusion. Provisions were therefore made for detailed study of this phenomenon in the present experiment through use of regression techniques.

Further data reduction was accomplished with the use of a PDP-12 computer. The program performed several operations. For each subject and session, interval histograms of the IRTs were derived by categorizing each response-response interval into one of 15 time bins. Since the present experiment was concerned with the temporal characteristics of rapid response sequences, maximum resolution of short IRTs was desirable. In order to accomplished this, histogram bin widths were set to 20 ms. The first 14 bins could therefore be used to categorize IRTs of less than 280 ms. Observations greater than 280 ms were accumulated in the 15th interval regardless of IRT duration. Several features of the computer derived IRT distributions were then quantified.

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The percent of IRT observations falling within the 280 ms limit was routinely calculated. This index provided a convenient method of precisely specifying the amount of response data being catagorized as a result of the fast IRT selection procedure. The occurrence of a well-defined peak in the distribution of fast IRTs permitted the identification of a modal response interval. This peak was identified by scanning IRT distributions to locate relative maxima within the first 14 intervals. It was further required that there be a dip in the frequencies of observations between the interval containing the modal fast IRT and the 15th bin which contained the "overflow" observations. These requirements ensured identification of an isolated peak IRT and not a simple buildup in response frequencies in the vicinity of the 15th interval. The midpoint of the IRT interval within which the cumulative five percent of the fastest IRT observations fell was arbitrarily selected as an index of the upper limits of responding. Observations corresponding to post-reinforcement pauses were not included when deriving the fastest five percent measure or when determining the percent of response data accounted for by the IRT analysis. Specifically, total reinforcements were subtracted from the total responses and the result used as a denominator in calculating the percent indices. This simple correction was appropriate since post-reinforcement pauses were considerably longer than any of the fast IRT observations.

Baseline values for each of the above performance variables were obtained by averaging measures obtained during the 10 days preceeding exposure to transfusion or surgical control procedures. For each dependent variable, behavioral recovery was defined as the number of days required for recovery performance scores to reach or exceed baseline levels.

A one-way analysis of covariance (BMDP1V) (11) was used to evaluate overall treatment effects. The analysis of covariance (ANCOVA) provides a method of evaluating and statistically controlling possible sources of experimental error. The linear regression analyses performed in conjunction with ANCOVA permitted study of the relationships between the selected covariates and dependent variables as a function of the experimental treatment conditions. In this experiment baseline values of the performance variables and the amounts of blood replaced were selected as covariates. Dependent variables were output ratios, fastest (cumulative 5 percent) IRTs, modal IRTs ( <280 ms), and the percents of response data falling within the range of the IRT interval histogram. ANCOVA was applied to the test scores obtained during the seven consecutive days following transfusion or surgical control procedures. Separate analyses were performed on recovery data, i.e., the days required for each dependent variable to return to baseline levels. The significance of differences between treatment groups were evaluated by using the pairwise comparison procedure provided with the BMDP1V program. The significance of within group differences in performance between baseline and initial (+24 hours) recovery sessions were evaluated by using t-tests for correlated data. The $P \leq 0.05$ level was used for all tests of significance.

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## RESULTS

Two rats died during surgical procedures. Fifteen of the remaining subjects were equally divided among control, BSA, and SFH conditions. The remaining four rats were transfused with the hemoglobin-albumin mixture. The average percent reductions (and ranges) in hematocrits for the three transfused groups were: BSA, 66.4 (65-68); SFH, 65.6 (63-68); MIX, 69.5 (67-75). Generally, animals were active and appeared alert within a few hours of transfusion and there were no obvious differences in the appearance or behavior of subjects immediately before operant testing 24 hr after treatment.

### Response Rates

The 8 week training period was sufficient to produce high, stable response rates in all experimental groups. The average baseline rate for all groups was 3177 responses per 30 min session. The average 30 min baseline response rate and corresponding range for each group was as follows: Controls, 2897 (2417-3706); BSA, 3154 (1668-4566); SFH, 3722 (2362-5455); MIX, 2875 (2491-3591). The difference between baseline group means were not significant. The 24 hr recovery response rates for SFH, BSA, and MIX groups were all significantly less than their respective baseline rates based on t-tests for correlated data. The response rate of the surgical control group did not differ significantly from the baseline rate.

The initial (24 hr) effects of each experimental condition on output ratios was significantly influenced by baseline response rates. In addition, for transfused rats the behavioral effects of the transfusates were significantly related to the amounts of blood replaced. The influence of the baseline response rate covariate was most dramatic during the first post-treatment test session. The slope of the linear regression equation relating this covariate to the 24 hr output ratios, was 15.90 ± 4.45 percent S.E. per 1000 baseline responses. This relationship did not differ significantly between groups. Thus the recovery performance of a rat (or group) with a baseline level of 4000 responses would be approximately 16 percent better than a subject (or group) with a base rate of 3000 responses regardless of the experimental treatment condition. The influence of this covariate necessitated the adjustment of the +24 hr group means in order to compensate for group differences in baseline performance. The response rate covariate did not significantly influence recovery performance beyond the +24 hr test session. In contrast to this finding, the blood replacement covariate significantly influenced performance through the fifth daily recovery session. At 24 hrs there was an additional 5.93 ± 2.20 percent S.E. drop in performance for each additional one percent increase in the amount of blood replaced. By the fifth day this coefficient had declined to approximately three percent. There were no significant differences in the blood covariate regression coefficients between transfusion groups. Adjustments of group means to correct for difference in average blood replacement levels did not appreciably affect the relationships between the BSA and SFH groups since they were closely matched with respect to

this variable. The corrected relative performance of the MIX group was improved with respect to BSA and SFH since the MIX group had received a greater average blood replacement.

Baseline response rates were not significantly related to the time required to reattain baseline performance following transfusion or surgical control procedures. However, blood replacement did influence recovery times. A delay of approximately 0.488 days ± 0.28 days S.E. was added for each one percent decrease in hematocrit.

ANCOVA of the output ratio data are presented in conjunction with Fig 1. In this figure output ratio data are corrected for the influence of performance and blood replacement covariates. Individual data points represent group averages and group codes are identified in the legend provided with the illustration. The results of the ANCOVA showed that the output ratios of all transfused groups were significantly depressed in comparison to the surgical control group 24 hr after transfusion. During this initial session the SFH group also made significantly fewer responses than either the BSA or MIX animals. Differences between control and MIX curves illustrated in Fig 1 were not statistically significant after the first day. The performance of BSA transfused animals was lower than the control group through the third post-transfusion session. The output ratios of the SFH group remained significantly lower than control and MIX groups through the 4th day of testing. Differences between the BSA and SFH groups illustrated in Fig 1 were significant during the 1st and 4th days and approached significance ($P = 0.0511$) on the second day. The output ratio recovery time in days (not illustrated) of the SFH transfused group (7.5 ± 1.2 S.E.) was significantly longer than that of control (1.75 ± 1.03 S.E.) and MIX (2.76 ± 1.43 S.E.) groups. The BSA group required 4.68 ± 1.09 S.E. days to recover baseline response rates and did not significantly differ from any of the other groups.

IRT Distributions

All subjects developed well-defined peaks in their interval histograms of inter-response times. The peaks of the IRT distributions were typically well within the 280 ms analysis range used in the present study. An average of 62 percent of the IRT observations (less post-reinforcement pauses) fell within the 280 ms range. The average modal value of the fast IRT distributions was 154 ms and the mean of all cumulative 5 percent cut-off points was 113 ms. The temporal locations of the modal and fast IRT portions of the IRT distributions were often found to stabilize before response rates. Therefore, although the proportion of observations falling within the 280 ms analysis interval increased with training, the location of the modal fast IRT was likely to remain unchanged.

Fig 2 illustrates the baseline and recovery IRT distributions of four transfused subjects. In this figure, data points of each IRT distribution are plotted as proportions of total responses (less IRTs associated with post-reinforcement pauses). This method of presentation facilitates comparisons among IRT distributions independent of the total numbers of IRT observations. The uppermost distribution for each subject, identified as "Base", is the normalized averaged IRT distribution derived from the 10 baseline sessions. The lower five distributions

are from the five consecutive recovery sessions of each subject. Data points are located at the midpoints of successive 20 ms intervals. The range of values covered in Fig 2 is approximately 280 ms. The treatment condition for each subject is given immediately below the letter identification code of each rat.

In the present experiment it has been established that there were considerable reductions in total responses following transfusion. The data presented in Fig 2 show that fast IRT peaks corresponding to epochs of fast responding are still present although characteristics of the distributions are changed. The IRT distributions of subjects H and S in Fig 2 are typical of many of the transfused subjects. For rat H, BSA transfusion produced a 35 percent drop in mean response rate 24 hr after treatment. However, the percent of IRTs falling within the analysis range only dropped from 81 percent to 76 percent. The relative stability of the normalized IRT distributions of this subject is evident in Fig 2 where the most obvious change is the shift to the right of the entire distribution. Three days were required for the mode of the IRT distribution of rat H to return to the baseline values. The treatment related changes in the normalized distributions of rat S are similar to those of rat H, although somewhat more pronounced. For rat S, the proportion of IRTs less than 280 ms dropped from 66 percent to 52 percent 24 hr after MIX transfusion and the modal fast IRT slowed 40 ms. Four days were required for the modal IRT of this rat to return to baseline values and nine days for the proportion of fast IRTs to reach baseline levels. Rats B and T were among the worst performers following treatment. The total responses emitted by rat B dropped 92 percent, 24 hr following MIX transfusion. Total responses for rat T declined 74 percent, 24 hr after SFH transfusion. In spite of these marked changes in overall response rates, both animals were capable of producing responses at moderately high rates as evidenced by the well-defined peaks of the IRT distributions of Fig 2. However, the characteristic shift in the modes of the distributions are more pronounced in these subjects, and longer recovery times are evident. The modal IRT for rat T returned to normal six days after transfusion, two days after the proportion of IRTs falling within the analysis interval returned to baseline levels. For rat B the proportion of IRTs faster than 280 ms returned to baseline within 4 days and total responses recovered by the sixth day. However, the mode of the fast IRT distribution for rat B had still not returned to its baseline value at the end of the 10 day recovery period.

Based on the results of the within group t-tests for paired observations, the slight changes in IRT distribution characteristics occurring 24 hr after surgical control procedures were not significant in comparison to baseline values. In contrast to this finding, transfusion produced significant + 24 hr reductions in the proportions or IRTs falling within the analysis range and significant changes (slowing) in the modal and fast IRT indices. However, the analyses of covariance performed upon the dependent variables derived from the IRT distributions disclosed few significant differences between transfusion conditions.

The proportions of IRTs falling within the 280 ms analysis range dropped approximately 20 percent (range, 19-21 percent) in comparison

to baseline values in transfused groups and improved slightly (5 percent) for the surgical control group. Overall group differences, observed 24 hr after treatment, were significant ($F_{3,14} = 5.05$, $P = 0.0140$). Although all transfused groups were significantly different from control subjects, there were no significant differences between transfusion groups. Similar relationships were observed between control vs. transfusion throughout the seven day recovery period, but differences between groups were not statistically significant. Approximately 5 days were required for the proportions of fast response IRTs to return to normal for transfused subjects. Recovery times were not differentially affected by transfusion conditions. The results of the analyses of covariance also demonstrated significant relationships between covariates and recovery changes in the proportions of fast IRTs. Relative shifts toward slow IRTs were less pronounced in subjects who had established high proportions of fast IRTs. IRT shifts were slightly greater blood replacements although the effects of this covariate were less important in comparison to characteristics of the IRT distribution before transfusion. Neither covariate appeared to be related to the recovery times of transfused animals.

The 24 hr recovery modal IRT of transfused groups slowed 24 ms (16 percent) in comparison to baseline levels ($t = 2.54$, $P < 0.025$). All transfused groups were slower than the control rats through the third recovery session. There were, however, no significant differences between transfusion groups or between transfusion groups and the control group during each of the seven recovery sessions. Changes in modal IRTs did not appear to be related to baseline values of the same variable. Amounts of blood replaced were directly related to shifting of the modal IRTs. This relationship was the greatest during the 2nd recovery session where each 1 percent decrease in hematocrit produced an additional 6.94 ms slowing the modal IRT. Recovery times for modal IRT data obtained from transfused rats were unexpectedly long, and in some cases extended beyond the 10 day recovery period during which this variable was routinely quantified. Since accurate recovery data were unavailable for some subjects, it was not possible to statistically evaluate recovery of modal IRTs.

The average value for the cumulative fast 5 percent IRT variable increased 20 ms or 17 percent over baseline values 24 hrs after transfusion ($t = 3.53$, $P < 0.01$) compared to a 1 ms (0.8 percent) increase in the control group ($t = 0.01$, $P > 0.05$). Overall group differences failed to reach significance ($F = 2.40$; d.f. = 3,14; $P = 0.1114$) during the 1st post-treatment session. Specific comparisons of the 24 hr group means showed that the greatest differences were due to the relative slowing of the BSA ($P = 0.0590$) and SFH ($P = 0.0230$) groups in comparison to surgical control animals. Significant overall group differences were observed during the 2nd and 3rd sessions. All transfused groups were slower than the control group on day two. BSA and SFH groups were slower than control and MIX groups during the 3rd test session. There were no significant differences between groups after the 3rd recovery test session. There were no significant differences between groups after the 3rd recovery test session. The effects of experimental conditions were not influenced by baseline fast 5 percent IRT levels, but were strongly influenced by percent reduction in hematocrit during transfusion. The influence of blood replacement level was greatest 24 hr after transfusion. During the 24 hr session, fast IRTs were slowed an additional

5 ms for each 1 percent reduction in hematocrit. The influence of the blood replacement covariate was statistically significant through the 5th day of testing. Appropriate analyses of fast 5 percent IRT recovery data were prohibited due to the failure of some transfused subjects to return to their baseline performance levels.

## DISCUSSION

A general pattern of performance changes was observed in all groups of transfused rats. Specifically, there were reductions in mean response rates, increases in the relative proportions of long IRTs and a slowing of IRTs representing fast response sequences. All of these factors contributed to the overall reductions in response rates following transfusion. The results also showed that transfused rats were still capable of organizing responses into high rate sequences although such episodes were slower and probably of shorter duration than comparable baseline activity. Interruptions of responding and increases in the durations of post-reinforcement pauses were the principal factors underlying the marked reductions in mean response rates. This explanation is supported by treatment related changes in IRT distributions and through direct observation of the performance of individual rats. Treatment related disruptions in well established patterns of FR behavior have been observed by other investigators. Weiss and Gott (4) proposed that these disturbances and changes in post-reinforcement pauses are the principal source of variability in FR experiments.

The results of the present study show that even those IRTs representing maximum response rates were affected by transfusion. This result differs from published reports (2,4,5,7,8) suggesting that this feature of FR performance is relatively unaffected by treatment variables. The results of the present study do not provide a clear explanation of this descrepancy. These differences may be due to properties of the transfusates and to blood replacement levels which produced unusually severe effects. Similar behavioral changes could perhaps accompany more extreme manipulation of other treatments variables. Alternatively, there may be a basic qualitative difference between the effects of transfusion and effects obtained following other experimental manipulations.

The results of the present experiment also demonstrate strong relationships between pre-treatment mean response rates and effect of transfusion materials. During the initial (+24 hr) test session the variation in output ratios related to pre-treatment response rates was in fact greater than that due to treatment effects. Such rate-dependent effects have been commonly observed in drug studies (12-14). Frequently, there is an inverse relationship between baseline and recovery response rates, i.e., performance of high-rate tasks is suppressed and performance of low-rate tasks is enhanced as a result of treatment effects. The data presented in this report do not support this general finding. Rats with the highest pre-treatment performance were least affected by surgery or transfusion, while the performance of those subjects who were less proficient were most severely disrupted. Had this source of

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variability not been recognized and properly controlled, the interpretation of the results could have been different since apparently non-significant pre-treatment group differences were translated into substantial post-transfusion effects. Moreover, the effects of individual differences in performance were not overcome by expressing data as a percent of baseline values since changes in output ratios were directly related to the pre-treatment performance levels.

Reduction in mean response rates and changes in IRT distributions followed exchange transfusion with each of the materials used in this study. In addition to these general effects, there were differential effects which depended upon the amount and type of transfusion solution employed. The amount of blood replacement and, therefore, oxygen carrying capacity of the blood, was directly related to the degree of behavioral change under all transfusion conditions. The degree of exchange transfusion was most strongly related to recovery of performance (in contrast to baseline performance, which was related to initial effects of transfusion). The relatively strong influence of this variable may have been peculiar to the extensive replacements used in this study. Further studies will be required to define precisely the relationships between extent of blood replacement and operant behavior and to determine the mechansisms of behavioral affects.

In the present study, all transfused groups were significantly worse than surgical control animals 24 hr after treatment. Animals transfused with MIX were least affected although the differences between this group and the BSA group were not significant. Rats transfused with SFH showed the greatest initial response decrement. The recovery time of the SFH group was also significantly longer than those of the control and MIX groups. The apparently poorer performance of the SFH group must be interpreted with caution for the following reasons. First, SFH was nearly completely excreted at the time of initial behavioral testing. Second, the performance of the MIX group which also received hemoglobin was virtually identical to the group transfused with BSA alone. Finally, the stroma-free hemoglobin solution which was used was an experimental formulation. Changes in the methods of preparation or administration might result in different behavioral effects. Therefore, it would be desirable to reevaluate SFH solutions as new variations are made available.

Tentatively, it might be proposed that tissue hypoxia following hemodilution accounted for some of the dose dependent transfusion effects observed in this study. Hypoxia is believed to depress food and water consumption through modification of the brain neurochemical mechanisms mediating appetite behavior (15). In the present study, reduced motivation to obtain food could account for lower response rates during performance of the food reinforced operant task following exchange transfusion. The behaviorial effects of hypoxia are, however, complex and hypotheses formulated exclusively on the basis of hypoxia are not cogent in explaining rate dependent effects or differences between transfusion groups. The additional behavioral deficit observed in the SFH group could be related to transient hypovolemia or hypoproteinaemia following the rapid excretion of large amounts of extraerythrocytic hemoglobin.

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The mechanisms of behavioral affects following transfusion will be studied in future investigations.

## RECOMMENDATIONS

Additional studies should be conducted to examine parametrically the behavioral effects of massive transfusion with standard crystalloids, colloids, and banked whole blood.

Dose-response studies should be initiated, in which changes in behavioral indices will be related to amounts of blood replaced. The resulting data would be useful for comparative evaluation of resuscitating solutions and procedures.

Behavioral testing should be extended to include evaluation of both more elementary and more complex forms of behavior following transfusion. The use of alternatives to food reinforcement should be explored in future operant conditioning experiments.

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## APPENDIX A

## FIGURE CAPTIONS

Fig 1 Recovery of FR response rates. For each recovery session the response score for each rat was expressed as a percent of the corresponding baseline rate. Figure 1 illustrates the group averages of the percent response scores obtained daily during a one week recovery period. Each data point has been adjusted to compensate for the influence of the baseline performance and blood replacement covariates.

Fig 2 FR interresponse time (IRT) distributions. Elapsed times between successive responses (IRTs) were measured to the nearest ms. Histograms were then obtained by depositing individual observations into one of 14 successive 20 ms wide intervals. A separate IRT distribution was obtained from each rat during each 30 min session. Distributions obtained from four rats are illustrated in Figure 2. The "base" plot represents the average of 10 consecutive baseline sessions. Individual IRT distributions obtained during five successive recovery days are presented below the "base" distributions. Each data point represents the proportion of data falling within each interval. The location of the midpoint of each 20 ms interval is given immedicately below the "+5" day distribution. A separate proportion and time scale is also provided as an inset.

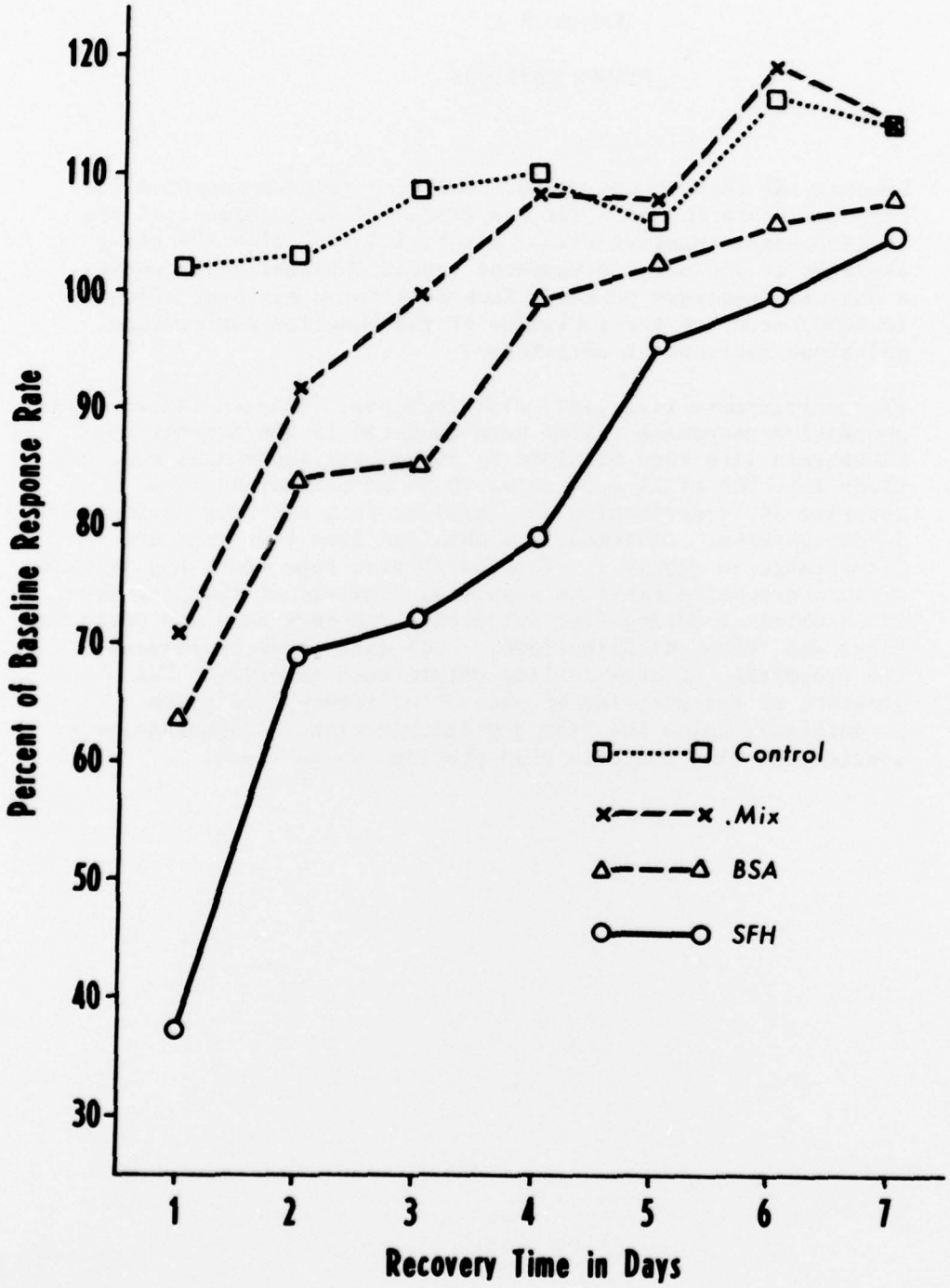
120
110
100
90
80
70
60
50
40
30
Percent of Baseline Response Rate
1
2
3
4
5
6
7
Recovery Time in Days
Control
Mix
BSA
SFH

Figure 1

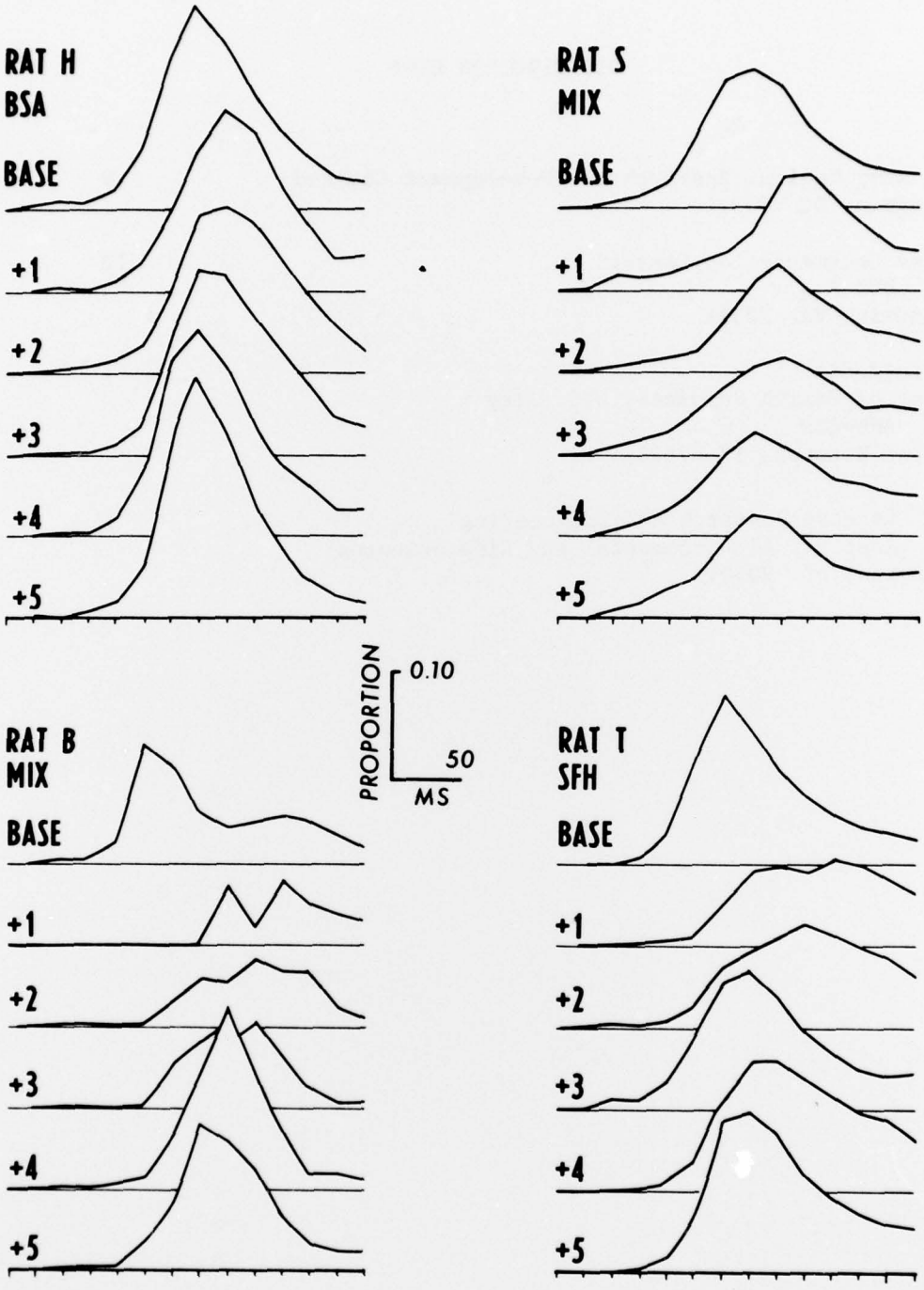
RAT H
BSA
BASE
+1
+2
+3
+4
+5
RAT S
MIX
BASE
+1
+2
+3
+4
+5
PROPORTION
0.10
50
MS
RAT B
MIX
BASE
+1
+2
+3
+4
+5
RAT T
SFH
BASE
+1
+2
+3
+4
+5

Figure 2

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