TRACE VERSUS DELAYED CONDITIONING OF HEART RATE IN RATS AS A FUNCTION OF US INTENSITY: A THESIS

By

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A DISSERTATION

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the degree of Master of Science

January 1968

APPROVED:

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ACKNOWLEDGEMENTS

The author would like to express his deep appreciation for the assistance and encouragement given by Dr. Robert D. Fitzgerald, under whose direction this research was conducted. In addition, the author is grateful to Drs. F. Robert Brush, David S. Phillips, Charles E. Phoenix, and Richard F. Thompson for their suggestions and creative criticism concerning certain aspects of the experiment.

T. J. T.

This paper is dedicated to my wife, Lisbeth.

T. J. T.

TABLE OF CONTENTS

INTRODUCTION

Classical conditioning may be viewed as a relatively simple and objective experimental method of studying learned changes in behavior. The traditional view of classical conditioning maintains that when a conditioned stimulus (CS) is paired with an unconditioned stimulus (US) on a number of occasions, an association is formed such that the CS now elicits a response called the conditioned response (CR), that it did not elicit before the pairings (Hilgard and Marquis, 1940; Kimble, 1961; Prokasy, 1965). The US according to this view must be able to evoke readily and consistantly an unlearned unconditioned response (UR), which is highly similar to the CR. The CS is defined as a neutral stimulus in the sense that the original response to the CS is assumed to be unlike the CR that subsequently develops.

One of the difficulties with this view of the conditioning process is that in several classical conditioning situations the CS elicits an original response which is similar in many respects to the CR. For example, investigators studying the galvanic skin response (GSR) typically present a series of CS alone trials to habituate an original GSR which is similar in form and magnitude to the conditioned GSR (Wickins and Harding, 1965). This same problem is present in the case of the heart-rate response (Fitzgerald, Vardaris, and Teyler, 1966), a reaction that was studied in the present investigation. However, it is important to realize that the occurrence of an original response to the CS that is like the CR does not necessarily indicate that conditioning

did not occur. Rather, the issue loses some of its significance if one views the pairing of the CS and US as one of the essential requirements for conditioning to occur. In discussing this problem of having the CR like the original response to the CS, Kimmel (1966) has reaffirmed the position taken by many investigators of the phenomenon (Fitzgerald, 1966; Grings, 1963) that the occurrence of conditioning should be judged in terms of the performance of an experimental group relative to that of a control group that does not receive forward pairings of the CS and US. Given this view of conditioning there is no reason why the CR could not be like the original response to the CS. This will be the position adopted in the present paper.

Although electric shock is the most commonly used US in aversive conditioning situations, intense sound, bright light, an air puff directed to the cornea of the eye, and acid applied to the mouth have also been employed. Pavlov (1927) observed that the effect of increasing US intensity was to increase the magnitude of the CR. As will be indicated below, the results of more recent studies, in most cases, agree with the findings of Pavlov.

In the typical conditioning experiment the CS begins a brief time before the onset of the US. The time between the beginning of the CS and the beginning of the US is called the CS-US interval. If the CS terminates before the onset of the US the procedure is termed trace conditioning. If the CS overlaps the US in time the procedure is termed delayed conditioning. Pavlov (1927) was the first investigator to study these variables and found that delayed conditioning generally resulted in a CR of a greater magnitude or probability than did trace

conditioning. Since the time of Pavlov this observation has been repeatedly confirmed, although the number of studies is small. Results of several recent studies investigating trace and delayed classical conditioning will be discussed below.

The broad purpose of the present investigation was to study the effects of trace and delayed conditioning on the heart-rate response in rats as a function of US intensity. The following is a summary of the pertinent experiments that have explored the effects of these two variables in classical conditioning situations.

Studies Varying US Intensity

Most of the evidence of the effects of US intensity in classical conditioning has come from human-eyelid conditioning studies. These studies have repeatedly demonstrated that the rate of acquisition and asymptotic level of the CR increase as the intensity of the US is increased (Burstein, 1965; Burstein, 1967; Passey, 1948; Prokasy, 1967; Ross and Hunter, 1959; Spence and Platt, 1966; Suboski, 1967). The general procedure used in all of the experiments consisted of pairing a tone or light (CS) with air puffs (US) of varying strengths to the cornea of the eye in a between subjects design.

There are several studies of autonomic nervous system conditioning that have looked at the effects of US intensity. Wickins and Harding (1965) investigated the effects of US intensity upon human GSR. A within subjects (Ss) design was employed, in which each S received two different CSs, each of which was paired with a US of a different intensity (1.5 or 2.5 ma. shock). Twenty trials were presented to each S

at a 1 sec. CS-US interval. They found that the magnitude of the conditioned GSR was greatest to the CS that was paired with the strongest US.

Some evidence is available to suggest that the relationship of increasing CR magnitude with increasing US intensity may also be present for classically conditioned heart-rate responses in humans. Bersh, Notterman, and Schoenfeld (1956), using a trace conditioning procedure with a 6 sec. CS-US interval and two values of US intensity (20 or 28 volts AC), found that after 11 paired trials the conditioned heart-rate response was greater in the group receiving the more intense US. There is also evidence in an experiment by Obrist, Wood, Perez-Reyes (1965), for this effect of US intensity on the human cardiac response. Each S received 16 pairings of a 2 sec. visual CS and one of two shock intensities (1.88 or 3.86 ma.). The group that received the more intense US exhibited a larger conditioned heart-rate response than the group receiving the less intense shock.

All of the studies cited above offer support for the notion that the strength of the CR increases as the intensity of the US is increased. One must note, however, that a basic problem encountered in all conditioning studies involving human Ss is the inherent complexity of the organism. For example, Hastings and Obrist (1967) argue that many of the human cardiac conditioning studies are confounded by the effects of verbally mediated instructional sets, and suggest that the resultant CR's may not be based on the traditional CS-US pairings. Perhaps, then, in an analysis of the effects of US intensity on conditioning it is more profitable to consider those experiments utilizing infrahuman Ss, in whom such sets would not be relevant factors.

In one of the few animal studies in which US intensity has been varied, three separate tone CS's were randomly presented to each dog (Dykman, Gantt, and Whitehorn, 1956). Associated with each CS was a US of a different intensity. Each of the four Ss received over 500 reinforced trials. The effect of US intensity on the cardiac CR was similar to that reported in the human literature in that the magnitude of the CR was greatest to the CS paired with the more intense US.

A contradictory finding was reported by Warstler and Ost (1965), who studied salivary conditioning in the dog. Three different concentrations of acetic acid (0.3, 1.5, and 7.5%) were used as the USs. The animals were given 10 reinforced trials per day for 10 days. The authors found that the CR magnitude of the high US group was less than that of the intermediate US group. The authors suggested that other studies of US intensity might have obtained an inversion had they used a very strong US.

A similar result was obtained in a recent study by Holdstock and Schwartzbaum (1965) in which the effect of two values of US intensity (0.5 and 1.5 ma. shock) on the conditioned heart-rate response of restrained rats was investigated. Each S received 80 acquisition trials during five days of training. These investigators found that the magnitude of the CR, which in this case was a deceleration in heart rate, was greater with the 0.5 ma. than with the 1.5 ma. US. Unfortunately, the method that Holdstock and Schwartzbaum used to present trials makes it difficult to draw any conclusions regarding their results. Their method consisted of giving the CS only when there was no struggling in a 5 sec. period prior to the time that the CS was scheduled to occur. Thus, CS

presentations were made contingent upon the absence of struggling. It is not possible to know what effect this procedure might have had on the outcome of their study.

To summarize, the results of most of the experiments cited above indicate that as the intensity of the US is increased the magnitude of the CR also increased, however, there are two notable exceptions to this general finding. In addition to its effect on the magnitude of the heart-rate CR in infrahumans, there is evidence to suggest that US intensity may also influence the direction of that response. By direction is meant whether the cardiac CR is an acceleration or a deceleration in rate relative to some baseline. Fitzgerald, Vardaris, and Teyler (1966), in a study involving 55 dogs, gave each subject 30 acquisition trials during two days of training. The US was a 9.9 ma. shock delivered to the hind quarters of the animal. The results indicated that the vast majority of Ss exhibited an accelerative CR. In a similar study involving 60 dogs, Fitzgerald (1966) presented each S with 12 acquisition trials and found that the predominant heart-rate CR was again accelerative.

Dykman, Gantt, and Whitehorn (1956), on the other hand, using a less intense shock of 1.8 ma. delivered to the front paws of the dog, reported finding a large number of Ss showing a decelerative CR. In a study by Lang and Black (1963), investigating the effect of three US intensities (3,4 and 8 ma.) applied to the paws of the dogs, more decelerative responses were observed at low shock levels than at high shock levels.

McDonald, Stern, and Hahn (1963), in a study using unrestrained rats and a 1.5 ma. foot shock to the S through a grid floor, presented

216 trials over 27 days and obtained results indicating that the CR was an acceleration in heart rate. Fehr and Stern (1965), in a study using unrestrained rats and a 1.5 ma. foot shock, presented the animals with 200 paired trials over four days. They obtained an accelerative CR. Black and Black (1967) also utilized freely moving rats and a 1.5 ma. foot shock. The animals received 30 paired trials in the single training session. The CR in this study was also a heart-rate acceleration.

Quite different results have been obtained in several other studies involving restrained rats. Fitzgerald, Vardaris, and Brown (1966), used a 1.8 ma. shock delivered across the chest of the animals. These animals received 42 paired trials over two days. In this experiment the CR was not an acceleration in heart rate but a deceleration. The experiment of Holdstock and Schwartzbaum (1966), using a 1.8 ma. US and restrained rats, obtained a deceleration in heart rate as the CR. These seemingly contradictory results could be due to the fact that the Ss in the former studies, showing accelerative CR's, were freely moving while in the latter studies the Ss were restrained. Conceivably this restraint on the Ss activity prevented skeletal responses from occurring which might have produced an acceleration in heart rate to the CS. An alternative explanation is that the restrained Ss struggled during the intertrial interval and with the onset of the CS stopped struggling. If this occurred a decelerative CR might be expected. However, as Fitzgerald, Vardaris, and Brown (1966) pointed out, struggling, in general, was infrequent in their experiment.

Black (1965) also agrees that "skeletal activity artifacts" do not appear to account for heart-rate conditioning in dogs. Obviously, the

results of the heart-rate conditioning experiments conducted under the paralyzing agent, curare, indicate that conditioning can occur in the absence of any skeletal activity (Black, 1965; Black, Carlson, and Solomon, 1962; Yehle, Dauth, and Schneiderman, 1967). Still other possible explanations of the variation in direction of the CR's are (1) that the subjective intensities of the US's may have differed in the restrained and unrestrained situations due to the different locations of the shocking electrodes and (2) that the higher resting heart rate of restrained Ss may have influenced the direction of the CR.

It will be recalled that in the studies using freely moving Ss a 1.5 ma. shock was delivered to the feet of the animals through a grid floor, while in the studies using restrained Ss a 1.8 ma. or 1.5 ma. shock was delivered to either the chest or the tail. It could be argued that the foot shocks were more noxious than either the chest or the tail shocks because of the high density of nerve endings in the feet relative to the chest or tail. Following this line of reasoning and recalling that Holdstock and Schwartzbaum found less deceleration in the group receiving the more intense US, we might expect the Ss receiving the more noxious foot shock to show an accelerative CR, and the chest and tail shock animals to show a decelerative CR. Moreover, had Holdstock and Schwartzbaum used a US intensity higher than 1.5 ma. it is conceivable that the heart-rate CR would have been accelerative. One purpose of this study is to explore this possibility by studying the effects of a wide range of US intensities on both the magnitude and direction of the heart-rate CR in rats.

Studies Comparing Trace and Delayed Conditioning

A second purpose of this study is to look at the effects of trace versus delayed conditioning on the magnitude and direction of the heart-rate response in rats. Church and Black (1958), investigating cardiac conditioning in the dog, used a 4.5 ma. shock delivered to the hind paws as the US. The factorial experiment included a 5 and 20 sec. CS-US interval and a trace and a delayed conditioning factor. The animals received only 10 acquisition trials. The results suggested that the use of delayed conditioning resulted in an accelerative CR that had a greater magnitude than the CR obtained using trace conditioning. The authors found that this difference was apparent only at the longer CS-US interval. While this observed difference did not reach statistical significance, one possible reason for this was the failure of the investigators to give the animals pre-training adaptation to the restraining device. Ommission of this procedure frequently leads to excessive struggling during the training period and corresponding variability in heart rate.

Black, Carlson, and Solomon (1962) conditioned dogs using a 4.5 ma. US delivered to the hindpaw of the Ss. The authors reported that the magnitude of the CR was greater for the dogs trained under a delayed procedure than those trained under a trace procedure. They also reported that the animals in the trace conditioning group gave more decelerative CR's (33%) than animals in the delayed group (1%).

Another of the few studies comparing trace versus delayed procedures is that of Schneiderman (1966). Several values of CS-US interval (0.25 to 2.0 sec.) were factorially combined with trace and delayed procedures

in this study of the rabbit's nicitating membrane response. The results indicated that the frequency of CR's was reliably higher under delayed conditioning than under trace conditioning for all values of CS-US interval.

Ellison (1964), on the other hand, studying salivary conditioning in dogs, found no differences between trace and delayed conditioning with an 8 sec. CS-US interval, but found delayed superior to trace at a CS-US interval of 16 sec. The author ascribed his results to the ineffectiveness of a trace procedure in producing a stable CR at long CS-US intervals.

Delayed conditioning has generally been shown to result in CR's of greater magnitude than has trace conditioning. In the case of heart rate there is also the suggestion that the direction of the response can be influenced by the use of trace or delayed procedures. In the investigation of the effects of these variables a factorial design was employed with trace and delayed groups for every level of US intensity so that any interactions between the US intensity factor and the trace versus delayed factor could be assessed.

Method

Subjects Two hundred and twenty-four experimentally naive, female, Long-Evans rats ranging in weight from 175 to 225 gm. served as Ss for this experiment. The Ss were obtained from Packard Farms, Beaverton, Oregon, and were housed under conditions of constant illumination and ad libitum food and water in the University of Oregon Medical School animal care facility. Two days prior to training, the Ss were placed in individual cages.

Apparatus The Ss were held in a commercially available restraining device consisting of a half-cylinder of plastic mounted on a flat platform. The half-cylinder had removable inserts at either end that could be positioned to hold the Ss securely without any apparent undue pain or discomfort. Typically, the rats readily entered the restraining apparatus and did not struggle appreciably during the testing period. The restraining device was located in a deactivated refrigerator shell to prevent extraneous sounds from reaching the Ss. This refrigerator was provided with a fresh air supply, a dim house light, and white noise (84 db sound pressure level relative to 0.0002 dynes/cm^2). A switching circuit was employed that made it possible to test two animals concurrently, each in a separate, isolated refrigerator. Trials were presented alternately to the two Ss.

The S's electrocardiogram (EKG) was recorded on a Grass model IIID polygraph by means of subcutaneous needle electrodes located on either side of the thoracic cavity. A sensitive microswitch was positioned on the EKG oscillograph pen to effect a contact closure on the R component of the QRS complex. This event in turn triggered a pulse-forming circuit

that provided pulses to the input of a Hewlett-Packard electronic counter. The counter in conjunction with a printing counter provided a printed record of the number of heart beats in discreet time intervals for each trial. Reliability of operation of the counting circuit was checked by displaying the output of the pulse former on the polygraph. In this manner the original EKG could be directly compared with the input to the counter-printer (Fitzgerald, Vardaris, and Teyler, in press).

Gross body movement was also recorded during the course of the experiment by means of a ceramic phonograph cartridge mounted under the rat restraining device, in such a way that the tip of the cartridge touched the base of the restrainer. When the Ss moved, the cartridge tip was displaced which in turn generated a small voltage. The output voltage of the phonograph cartridge was electronically integrated, resulting in a relay contact closure after a predetermined amount of movement had occurred. The closures occurring during predetermined periods for each trial were automatically printed out by a printing counter. The sensitivity of the integrating device was adjusted just below the level that prevented normal respiratory activity from being detected.

The US was a 1sec, 60 Hz, AC shock produced by a Foringer constant current stimulator and delivered through the EKG electrodes. An oscilloscope was used to monitor the various shock intensities during the course of the experiment. The CS was a 1000Hz tone, 16 db above the background noise present in the refrigerator. The duration of the CS for trace and delayed groups was 1 and 7 sec., respectively. The CS was generated by a Hewlett-Packard audio-oscillator, amplified, and presented through Wharfedale speakers. The CS-US interval was 6 sec.

The CS, US, intertrial interval (ITI), and CS-US interval were automatically timed and presented by means of Massey Dickinson transistorized programming modules. The experiment was conducted in one session of approximately 4 hours length.

Procedure At the start of the training session the pair of Ss to be conditioned were placed in the restraining devices and EKG electrodes attached. Each S received an initial 30 min. period of adaptation to the restraining device and testing chamber. During this time heart rate and movement samples were taken at 0,5,10 and 20 min. to obtain a measure of the degree of adaptation of the S to the environment. The Ss received no stimuli of any kind when these samples were taken. All Ss then received 20 CS-alone trials at an ITI of 70, 90, or 110 sec., with a mean of 90 sec. Following the CS-alone trials, all Ss received 30 acquisition and 30 extinction trials at an ITI of 160, 180, or 200 sec., with a mean of 180 sec. Extinction trials were identical to acquisition trials except for the omission of the US.

The Ss were divided into 16 groups of 14 Ss per group. Twelve of the groups were experimental and four were conditioning control groups. The 12 experimental groups represented six US intensities (0.4, 0.8, 1.2, 1.8, 3.0, and 5.0 ma.) with a trace and delayed group at each shock level. Of the four control groups, one pair received a 0.4 ma. US and the other pair a 5.0 ma. US. Within each pair one group received a 1 sec. CS corresponding to the trace experimental groups, and the other group received a 7 sec. CS corresponding to the delayed experimental groups. The presentation of stimuli for the control groups was identical to that of the

experimental groups with the exception that the CS followed the US by 70, 90, or 110 sec., with a mean of 90 sec. This interpolated US design was chosen as the best single control procedure for non-associative factors in the experiment.

The measure of conditioning was obtained on each trial by subtracting the number of heart beats occurring during the 6 sec. immediately preceeding the onset of the CS (pre-CS period) from the number of heart beats occurring during the 6 sec. CS-US interval (CS period). This measure will be referred to as the difference (D) score. In addition to the pre-CS and CS period heart-rate totals, a post US heart-rate measure was obtained by totaling the number of heart beats occurring in a 6 sec. period following the termination of the US. Since heart rate during the first second following the US could not be recorded due to technical difficulties, the heart rate during the next 5 sec. was multiplied by a constant of 6/5 to obtain a comparable value for comparison with the 6 sec. pre-CS and CS period values.

The data were punched on IBM cards for subsequent computer analysis.[1] The IBM cards were verified to insure that the cards were punched properly. An IBM 1130 with its associated soft-ware was used to analyze the data. The analysis of variance program included several checks on the source data so that missing or out-of-sequence cards could be detected.

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[1] The IBM data cards are available on request from the Department of Medical Psychology, University of Oregon Medical School.

Results

Adaptation - All Groups

Mean basal heart-rate levels in the four sampling periods of adaptation were fairly constant and similar for all groups. The mean heart rate of all Ss was 480 beats/min. with a range of group mean values from 465 to 495 beats/min. In an analysis of variance (Winer, 1962) of these results, the Groups effect and the Trials effect both failed to reach significance.[2]

Pre-CS Heart Rate

CS Alone and Extinction Pre-CS data are important from the standpoint that the index of conditioning is computed by taking into account the differences in heart rate between the pre-CS and the CS periods. It follows that a change in pre-CS values, CS values, or both, will affect the difference score. Although the pre-CS values did change during the course of the experiment, it will be shown that these changes cannot account for the conditioning data.

Pre-CS heart rate levels during CS alone are comparable for all groups. When averaged across the experimental groups, pre-CS heart rate increases from 471 beats/min. on the first two-trial block to 474 beats/min. on the last two-trial block ($F=4.01$, $df=9/1404$, $p < .001$). In extinction the pre-CS heart-rate levels averaged across the experimental groups decrease

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[2] The author would like to express his appreciation to Dean Bailey for assistance in the computer programming.

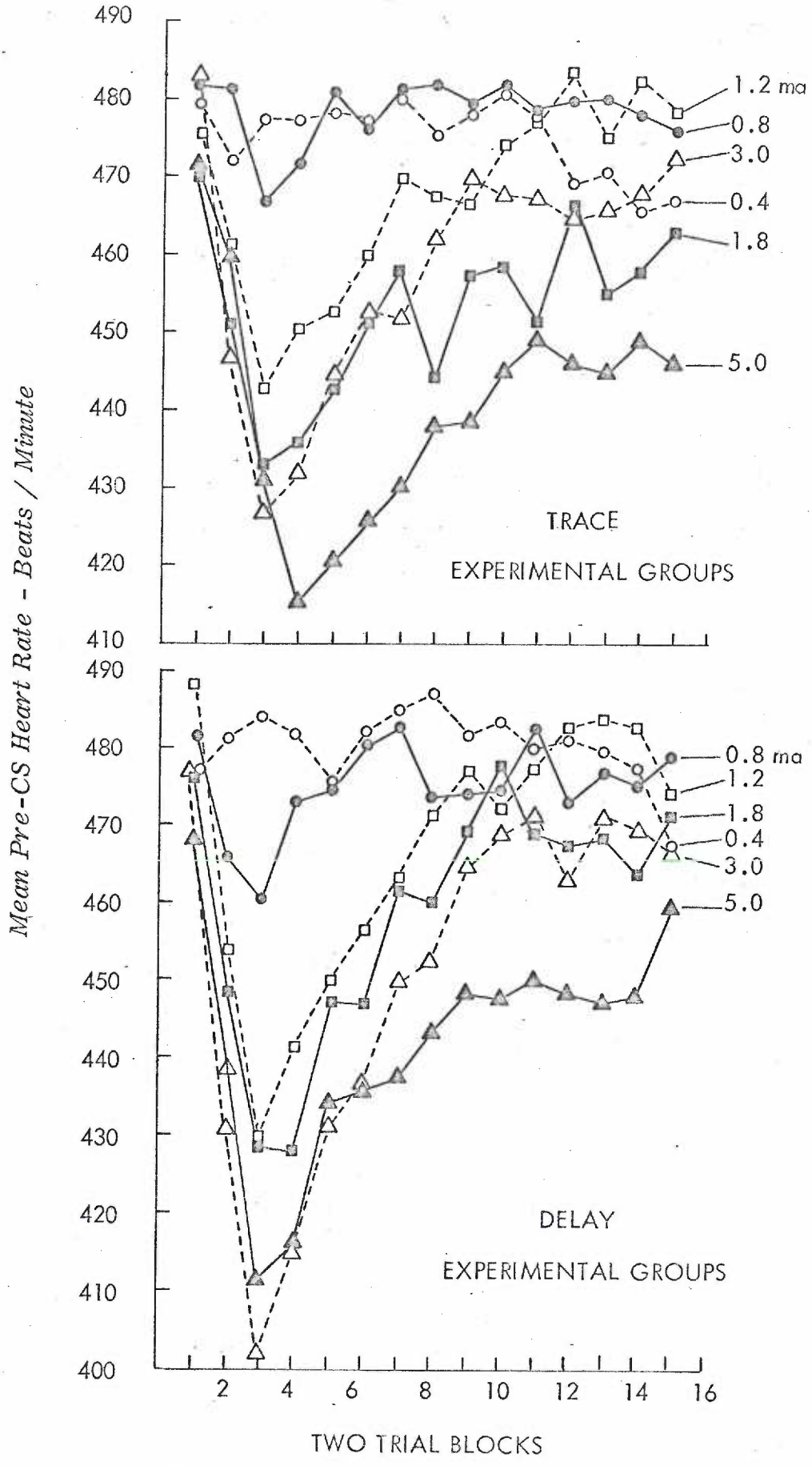
slightly from 470 beats/min. on the first two-trial block to 464 beats/min. on the last two-trial block (F=4.72, df=14/2184, $p < .001$). There were no group differences in pre-CS responding during CS alone or during extinction.

The results of the pre-CS analysis for the control groups in the CS alone and extinction phases of the experiment are similar to those of their matched experimental groups. Separate analyses of variance demonstrated that there are no significant differences between experimental and control groups in either CS alone or extinction. However, the Trials effect is reliable in both CS alone (F=3.13, df=9/936, $p < .001$) and extinction (F=2.64, df=14/1456, $p < .001$).

Acquisition Figure 1 shows the pre-CS heart rates for the experimental groups during successive two-trial blocks of acquisition. The upper part of the figure depicts the performance of the trace groups at the six US intensities and the lower graph depicts the performance of the delayed groups. It is clear from this figure that in contrast to the CS alone and extinction phases of the experiment, dramatic changes in pre-CS responding occur in acquisition for all groups except for those receiving the 0.4 ma. shock. These changes may be characterized as a large decrease in heart rate with the point of maximum decrease occurring on either the third or fourth two-trial block followed on later trials by a gradual increase toward the original baseline. The magnitude of the decrease appears to be a function of US intensity. An analysis of variance applied to these data resulted in a significant effect of Trials (F=37.26, df=14/2148, $p < .001$) and a significant effect of US Intensity (F=3.11, df=5/156, $p < .05$). The significant effect of US Intensity

Figure 1. Pre-CS heart rate in beats/minute during successive two-trial blocks of acquisition. The upper and lower graphs, respectively, depict the mean heart rate of the trace and delayed experimental groups at the six US intensities.

Figure 1


490
480
470
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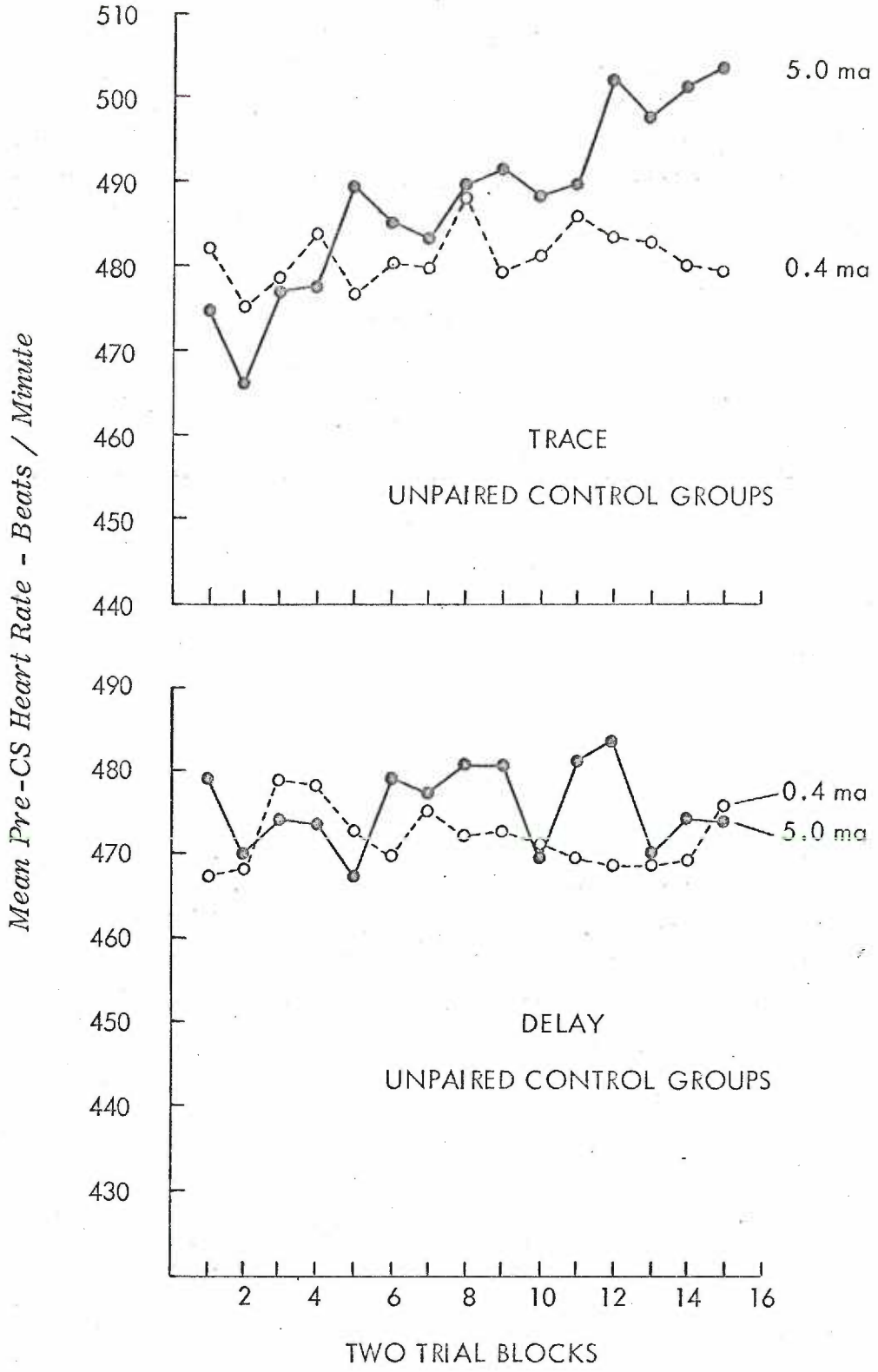
430
420
410
1.2 ma
0.8
3.0
0.4
1.8
5.0
TRACE
EXPERIMENTAL GROUPS
490
480
470
460
450
440
430
420
410
400
0.8 ma
1.2
1.8
0.4
3.0
5.0
DELAY
EXPERIMENTAL GROUPS
2
4
6
8
10
12
14
16
TWO TRIAL BLOCKS
Mean Pre-CS Heart Rate - Beats / Minute

indicates that higher values of US intensity result in increasing degrees of heart-rate depression. The analysis also resulted in a reliable interaction between Trials and Shock Intensity (F=4.21, df=70/2184, $p < .001$) and between Trials and Trace versus Delayed Conditioning (F=2.39, df=14/2184, $p < .01$). The interaction between Trials and Shock Intensity means that the rate of change of pre-CS responding over trials was reliably different at the various shock levels. The Trials and Trace versus Delayed interaction is a result of pre-CS heart rate changing at a greater rate for delayed groups as compared with the trace groups.

Figure 2 shows the pre-CS heart rate for the control groups in successive blocks of two-trials during acquisition. The upper and lower halves of the figure depict the mean heart rate of the trace and delayed control groups, respectively, at the individual US intensities. It should be noted that these pre-CS measures are not in the same temporal relationship to the US as are those for the experimental groups. This is because the interval between the US and CS for the unpaired control groups averaged 90 sec., whereas for the experimental groups it averaged 180 sec. It can be seen from Figure 2 that with the exception of the 5.0 ma. trace group, which shows an increase, there is little evidence that pre-CS heart rate changes systematically during acquisition. A 2x2x2 factorial analysis of variance comparing these data with those of the matched experimental groups showed a significant effect of Shock Intensity (F=5.65, df=1/104, $p < .05$), a significant effect of Experimental versus Control Treatments (F=5.32, df=1/104, $p < .05$), and a significant interaction between Shock Intensity and Experimental versus Control Treatments (F=6.89, df=1/104, $p < .05$). The analysis also showed a reliable effect of Trials

Figure 2. Pre-CS heart rate in beats/minute during successive two-trial blocks of acquisition. The upper and lower graphs, respectively, depict the mean heart rate of the trace and delayed control groups at the two US intensities.

Figure 2


510
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0.4 ma
TRACE
UNPAIRED CONTROL GROUPS
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480
470
460
450
440
430
0.4 ma
5.0 ma
DELAY
UNPAIRED CONTROL GROUPS
2
4
6
8
10
12
14
16
TWO TRIAL BLOCKS
Mean Pre-CS Heart Rate - Beats / Minute

(F=3.75, df=14/1456, $p < .001$), a significant interaction of Trials and Experimental versus Control Treatments (F=3.71, df=14/1456, $p < .001$), a significant interaction among Trials and Experimental versus Control Treatments and Shock Intensity (F=3.05, df=14/1456, $p < .001$) and a significant interaction among Trials and Experimental versus Control Treatments and Trace versus Delayed Conditioning (F=1.75, df=14/1456, $p < .05$). These outcomes are not unexpected if one recalls that the pre-CS levels of the 5.0 ma. experimental groups decreases whereas the 5.0 ma. trace control group increases, and the other groups demonstrate essentially no change.

CR Difference Score Data

Experimental Groups The mean CS minus pre-CS difference scores are presented in Figures 3a through 3f for each experimental group as a function of two-trial blocks in the CS-alone, acquisition, and extinction phases of the experiment. The two curves in each figure represent the heart-rate changes of the trace and delayed groups at the specified US intensities. An examination of the CS-alone data, plotted in the left-hand panels of the figures suggests that the initial heart-rate response to the CS is a dramatic deceleration that habituates over trials. An analysis of variance revealed that the effect of Trials was reliable (F=89.13, df=9/1404, $p < .001$) as was the interaction between Trials and Trace versus Delayed Conditioning (F=2.73, df=9/1404, $p < .01$). An inspection of the figures indicates that the interaction is due to the fact that the trace groups, in general, show a larger original response to the CS than the delayed groups and that responses made by the trace groups tend to habituate to

Figures 3a to 3f. Mean CS minus pre-CS difference scores in beats/minute for each experimental group as a function of two-trial blocks in CS alone, acquisition, and extinction. The two curves in each figure represent the difference scores of trace and delayed groups at the indicated US intensity.

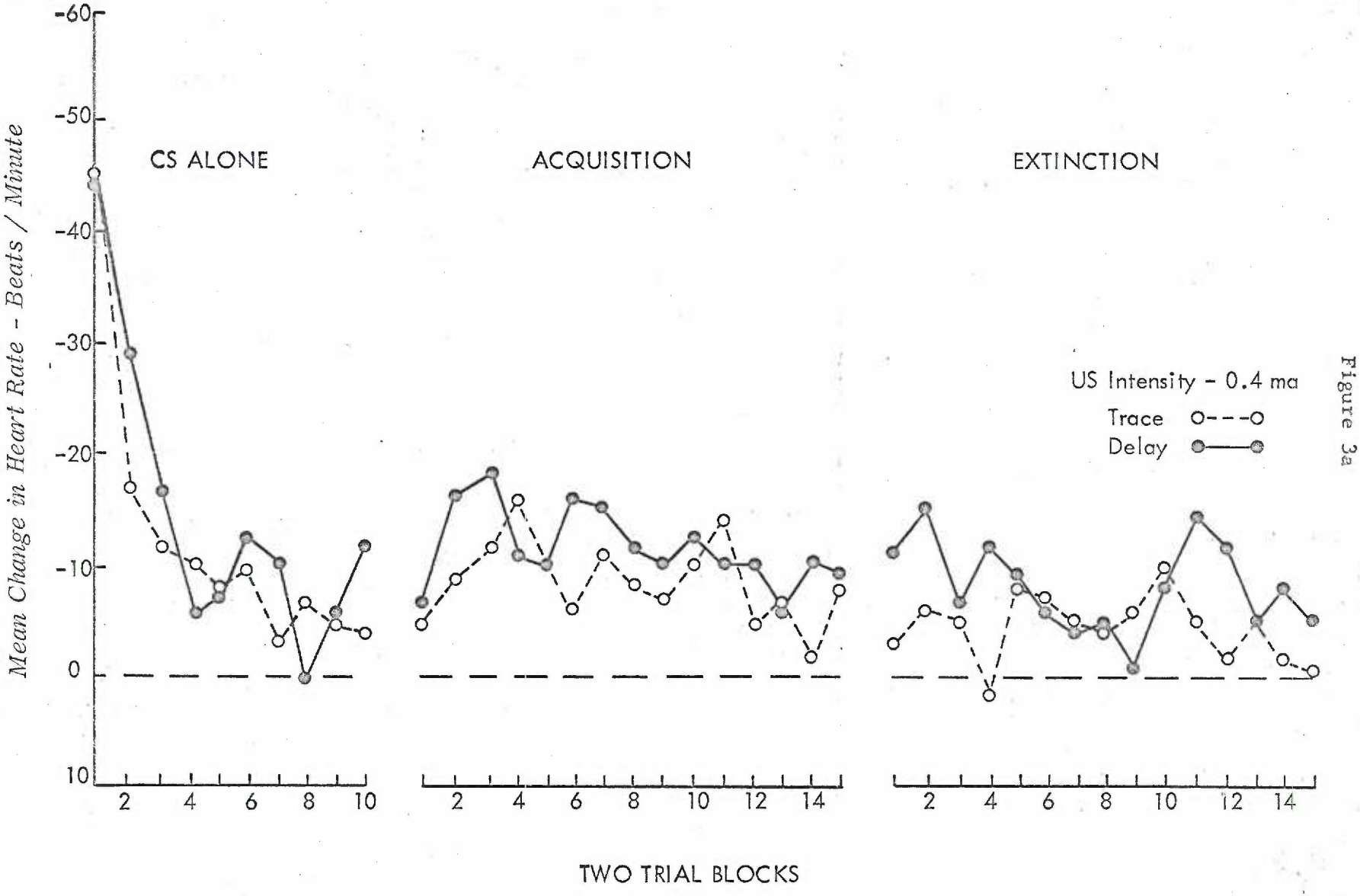
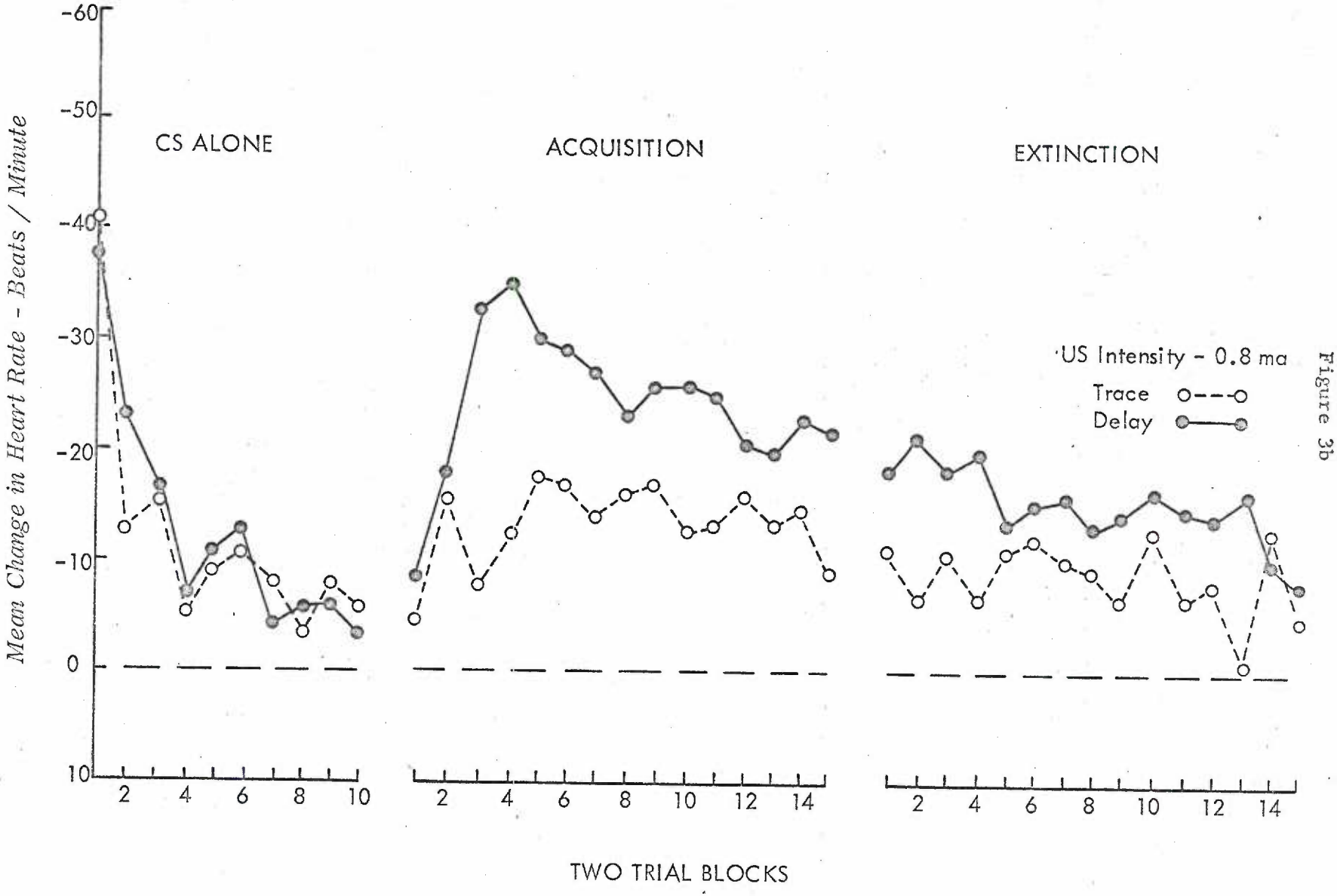

CS ALONE
ACQUISITION
EXTINCTION
Mean Change in Heart Rate - Beats / Minute
-60
-50
-40
-30
-20
-10
0
10
US Intensity - 0.4 ma
Trace
Delay
2
4
6
8
10
12
14
TWO TRIAL BLOCKS

Figure 3a


-60
-50
-40
-30
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-10
0
10
CS ALONE
ACQUISITION
EXTINCTION
US Intensity - 0.8 ma
Trace
Delay
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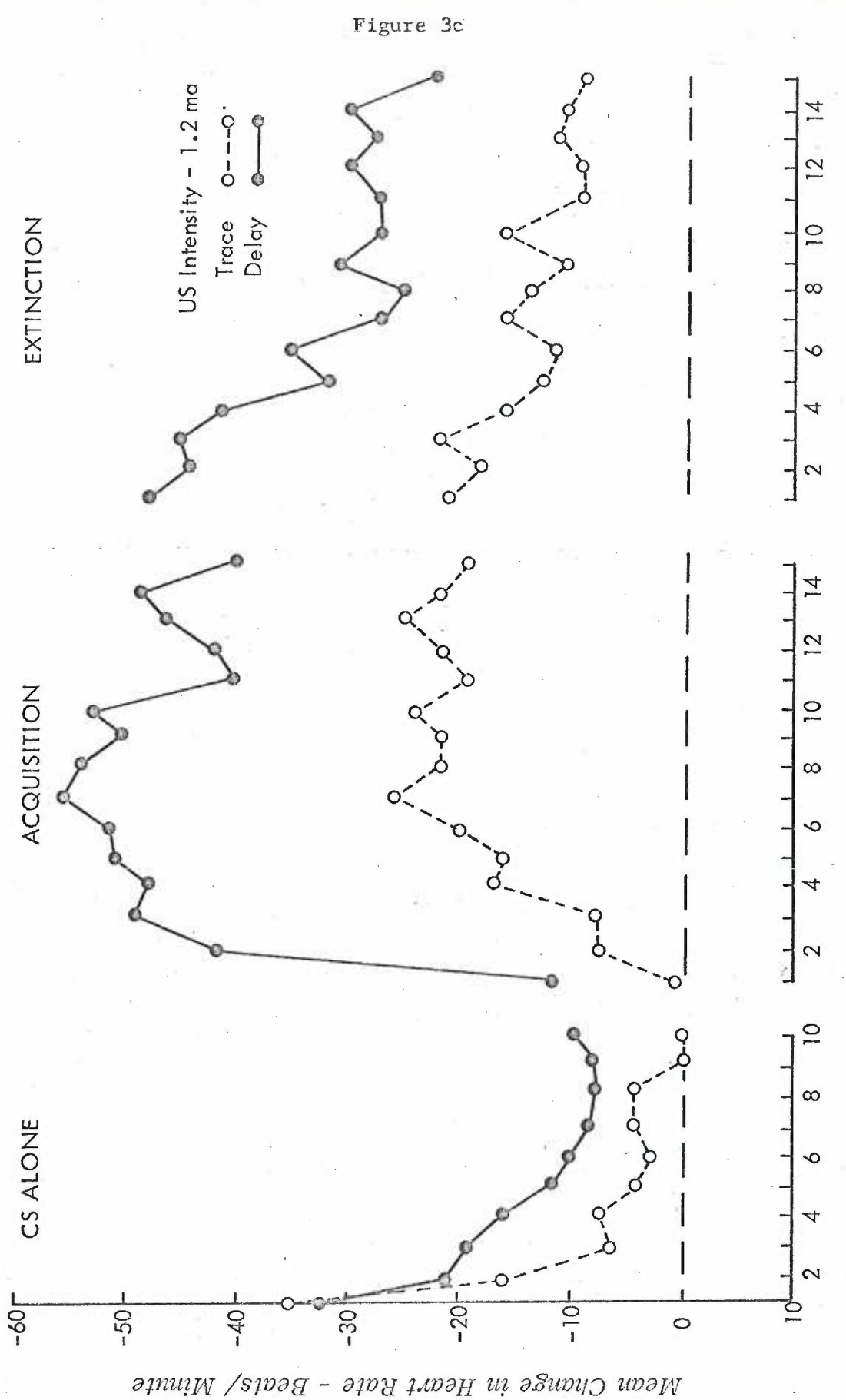
12
14
TWO TRIAL BLOCKS
Mean Change in Heart Rate - Beats / Minute

Figure 3b

Figure 3c


CS ALONE
ACQUISITION
EXTINCTION
US Intensity - 1.2 ma
Trace
Delay
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-40
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8
10
12
14
TWO TRIAL BLOCKS
Mean Change in Heart Rate - Beats/ Minute

Figure 3d

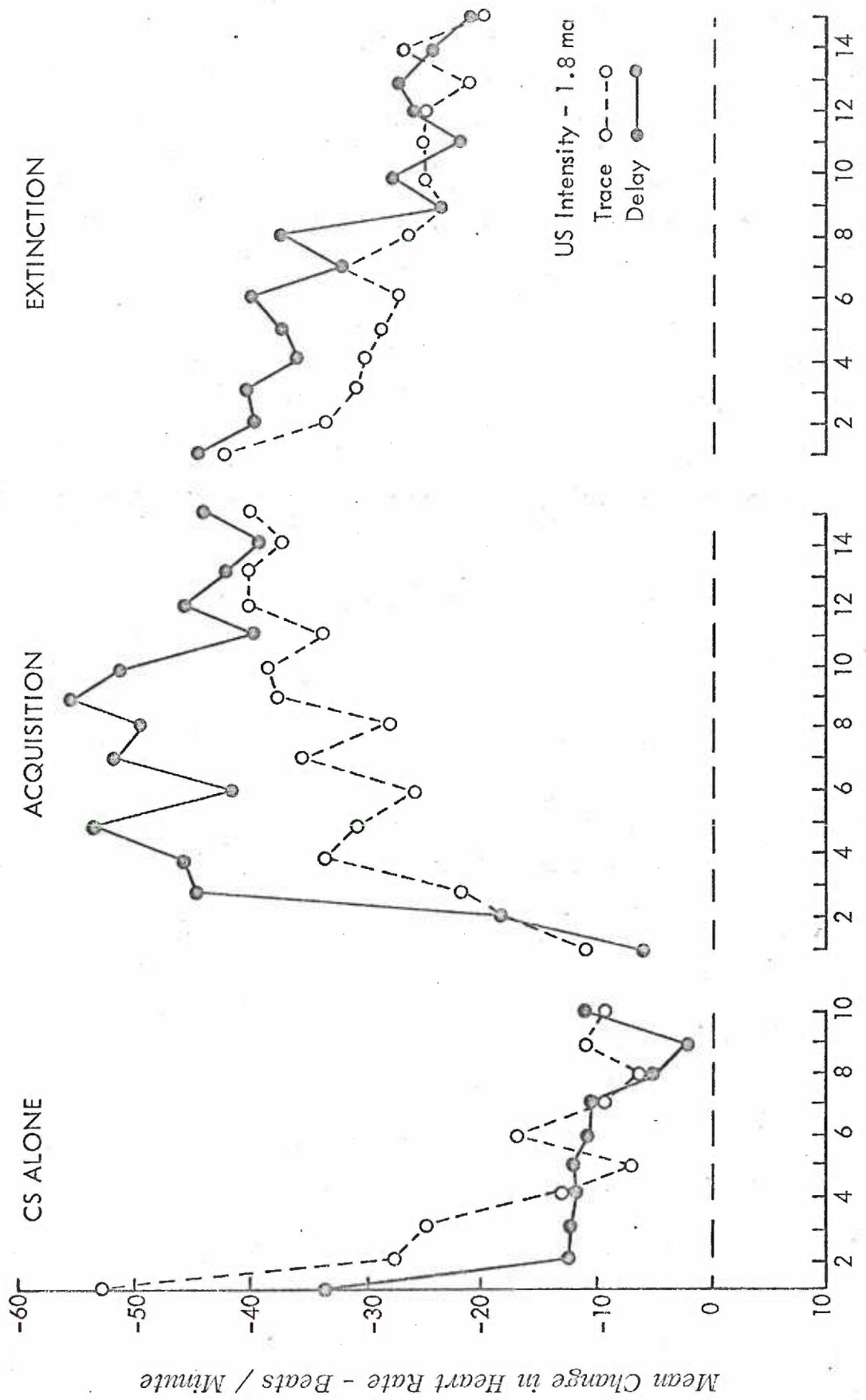

CS ALONE
ACQUISITION
EXTINCTION
US Intensity - 1.8 ma
Trace
Delay
-60
-50
-40
-30
-20
-10
0
10
2
4
6
8
10
12
14
TWO TRIAL BLOCKS
Mean Change in Heart Rate - Beats / Minute

Figure 3e

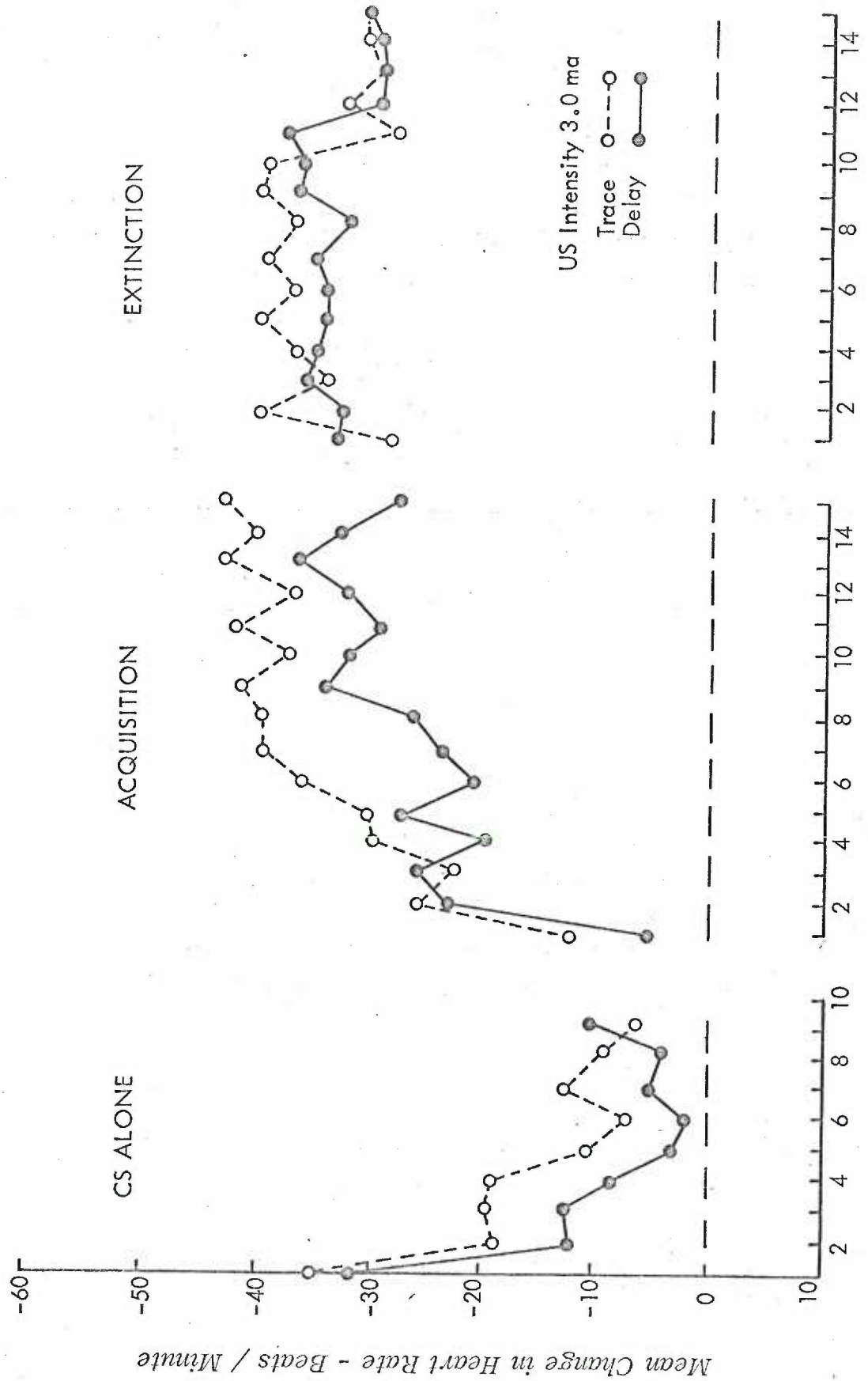
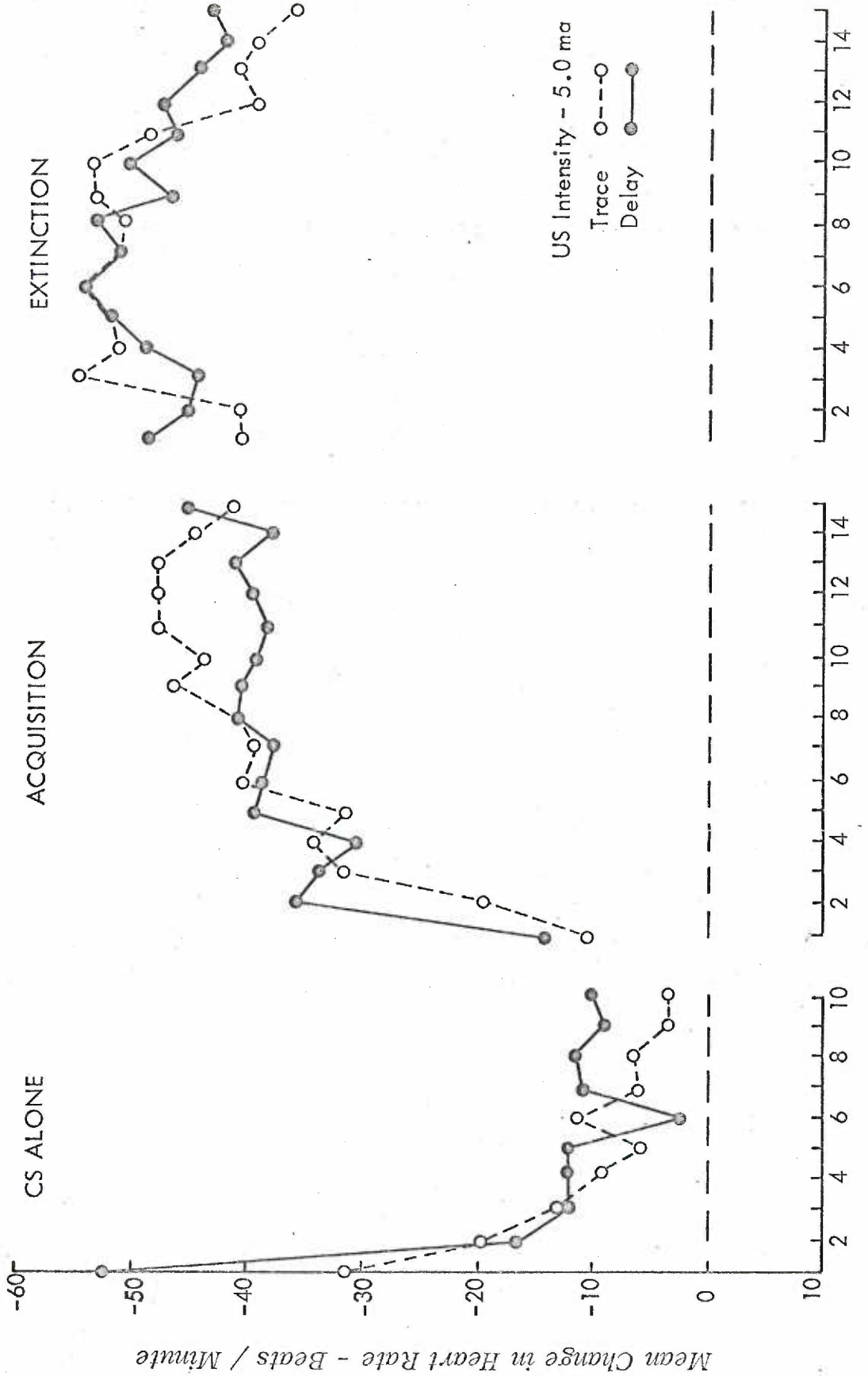

CS ALONE
ACQUISITION
EXTINCTION
US Intensity 3.0 ma
Trace
Delay
-60
-50
-40
-30
-20
-10
0
10
2
4
6
8
10
12
14
TWO TRIAL BLOCKS
Mean Change in Heart Rate - Beats / Minute

Figure 3f


EXTINCTION
ACQUISITION
CS ALONE
US Intensity - 5.0 ma
Trace
Delay
-60
-50
-40
-30
-20
-10
0
10
2
4
6
8
10
12
14
TWO TRIAL BLOCKS
Mean Change in Heart Rate - Beats / Minute

a level equal to or below that of the delayed groups.

The center portions of Figures 3a through 3f show the mean CR difference scores for each experimental group as a function of two-trial blocks in acquisition. An examination of these figures shows that the magnitude of the CR for the trace groups increases as a fairly linear function of US intensity. The CR magnitude of the delayed groups also shows an increase as a function of shock intensity, up to 1.2 ma. However, at 1.8, 3.0, and 5.0 ma. the CR magnitude of the delayed groups is less than at 1.2 ma. These figures also show that the magnitude of the CR for the trace groups is less than that of the delayed groups at all US intensities except the 3.0 and 5.0 ma. shock levels. Furthermore, the rate at which the CR developed seems to be greater for the delayed groups than for the trace groups at all levels of US intensity except 3.0 and 5.0 ma. These visually apparent differences were confirmed by an overall analysis of variance on the acquisition data. This test resulted in a significant effect of Shock Intensity ($F=8.72$, $df=5/156$, $p < .001$), a reliable effect of Trace versus Delayed Conditioning ($F=5.84$, $df=1/156$, $p < .05$), and a reliable effect of Trials ($F=21.69$, $df=14/2184$, $p < .001$). This test also resulted in significant interactions between Shock Intensity and Trace versus Delayed Conditioning ($F=3.00$, $df=1/156$, $p < .05$), between Trials and Trace versus Delayed Conditioning ($F=6.06$, $df=14/2184$, $p < .01$), and between Trials and Shock Intensity ($F=6.57$, $df=70/2184$, $p < .001$). The interaction between Shock Intensity and Trace versus Delayed Conditioning is due to the fact that the magnitude of the trace CR is less than that of the delayed CR at all but the highest US intensities. The interactions between Trials and Shock Intensity and between Trials and Trace versus Delayed Conditioning reflect the different rates of conditioning

in groups trained with trace or delayed procedures at different shock intensities, as a function of trials.

Another way of looking at these results is to examine the effects of trace versus delayed procedures and US intensity, ignoring the rate of conditioning. This is seen in Figure 4 which represents the mean CR data for the experimental groups averaged across acquisition trials as a function of US intensity. It can be seen from this figure that the CR magnitude of the trace groups increases monotonically with US intensity, whereas the magnitude of the CR in the delayed groups falls off at high shock levels. A similar computation involving only the last four acquisition trials revealed the same results. Separate F tests comparing trace and delayed groups at each US intensity show that the groups differ only at the 1.2 ma. level (F=14.90, df=1/156, $p < .001$).

The results of individual comparisons between all trace experimental groups and between all delayed experimental groups are presented in Table 1. A common line between groups indicates no significant differences between the groups. For example, the 0.4 ma. trace experimental group is not reliably different from the 1.2 ma. trace group but is different from the 1.8 ma. group. This table shows that the ordering of CR magnitudes is a linear function of increasing US intensity for the trace groups. The ordering of the delayed CR magnitudes, on the other hand, is not a linear function of US intensity above 0.8 ma.

Mean CR difference scores for the experimental groups during the extinction phase of the experiment are seen on the right-hand side of Figure 3a through 3f. An inspection of the performance of the 0.4, 0.8, 1.2, and 1.8 ma. trace and delayed groups seems to indicate that extinction performance is a function of terminal acquisition performance. The 3.0

Figure 4. Mean CR difference scores in beats/minute for each experimental group averaged across acquisition trials as a function of US intensity.

Figure 4

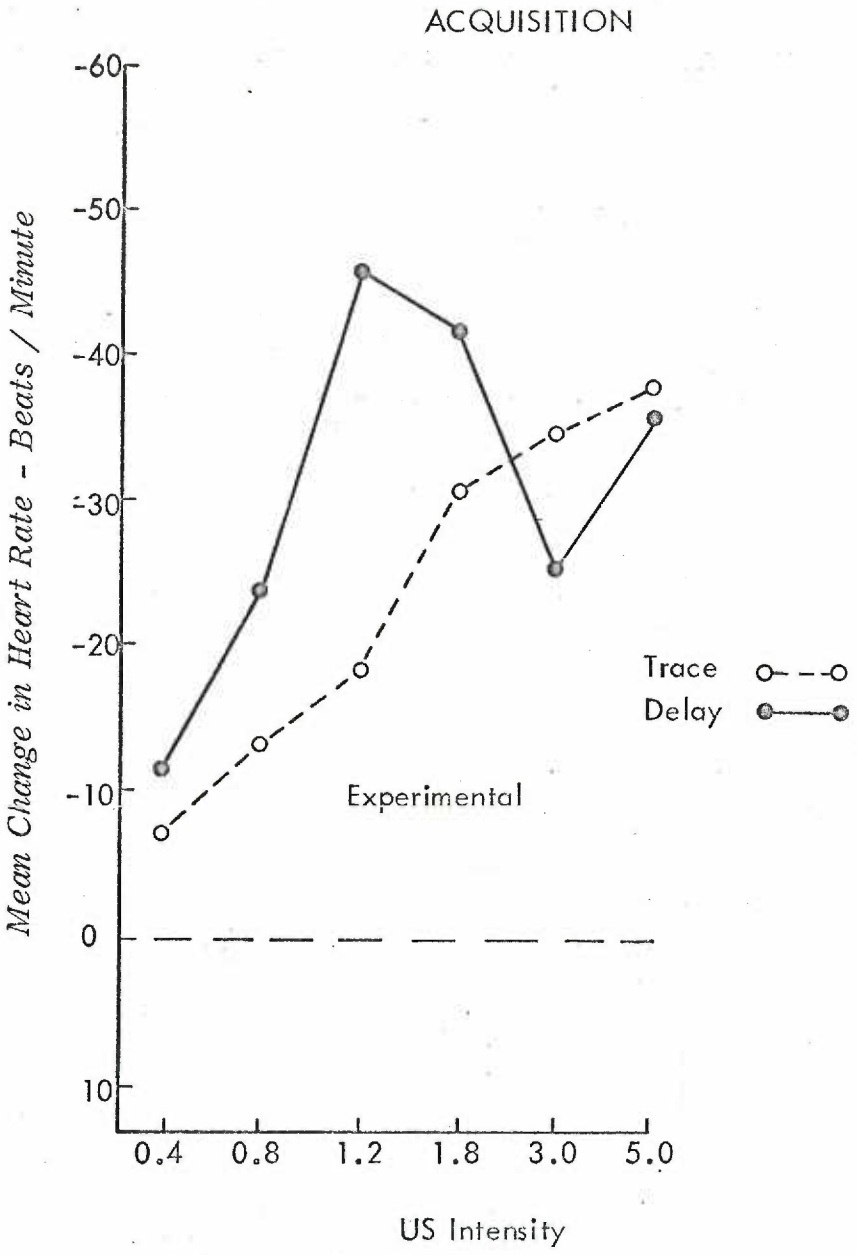
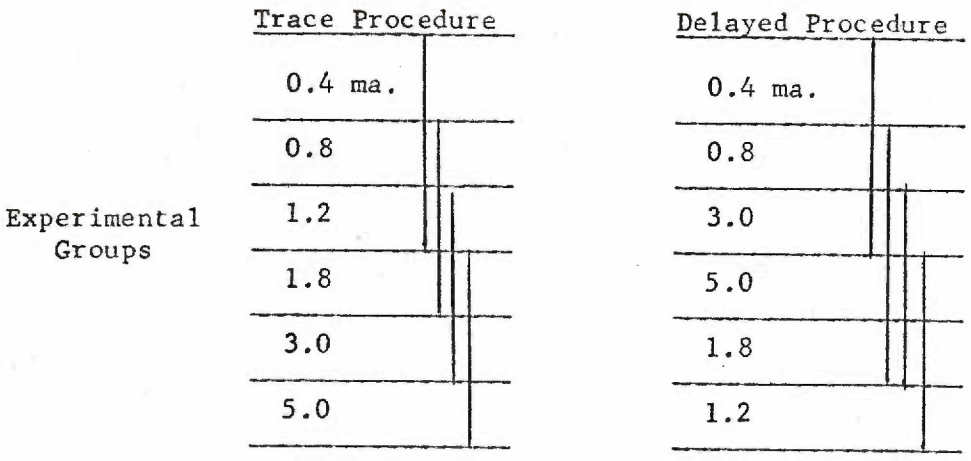

ACQUISITION
-60
-50
-40
-30
-20
-10
0
10
Mean Change in Heart Rate - Beats / Minute
Trace
Delay
Experimental
0.4
0.8
1.2
1.8
3.0
5.0
US Intensity

Table 1. Results of individual F tests on CR magnitude in acquisition for the experimental groups. A common line between groups indicates no significant differences.

Table 1



All significant differences are $p < .01$.

and 5.0 ma. trace and delayed groups (Figures 3e and 3f), however, exhibit little or no response decrement in the 30 extinction trials. An analysis of variance applied to these extinction data confirmed these observations. The test showed that the following effects are significant: US intensity (F=21.88, df=5/156, $p < .001$), Trace versus Delayed Conditioning (F=4.53, df=1/156, $p < .05$), Trials (F=7.09, df=14/2184, $p < .001$), and an interaction between Trials and Shock Intensity (F=1.61, df=70/2184, $p < .01$).

To determine whether the differences observed in extinction are a function of the starting point, i.e., the terminal level in acquisition, an analysis of covariance was performed on these extinction data, in which the performance on the last four acquisition trials was the covariate. This analysis resulted in a significant effect of the Covariate (F=81.34, df=1/222, $p < .001$), indicating that the terminal acquisition performance has a reliable effect on extinction performance. The analysis of variance of overall extinction performance was repeated with the effect of the covariance removed. The only effect of this procedure was to render the Trace versus Delayed effect nonsignificant. All other differences were the same as in the non-corrected analysis of variance except that the F ratios were reduced somewhat.

Control Groups Figures 5a and 5b depict the mean difference score data of the control groups during the CS alone, acquisition, and extinction phases of the experiment as a function of blocks of two-trials. It can be seen in the left-hand panels of the figures that the control groups exhibit a dramatic initial deceleration in heart rate to the CS that habituates with repeated presentations of the tone. This result

Figures 5a and 5b. Mean CS minus pre-CS difference scores in beats/minute for each control group as a function of two-trial blocks in CS alone, acquisition, and extinction. The two curves in each figure represent the difference score heart rate of trace and delayed groups at the indicated US intensity.

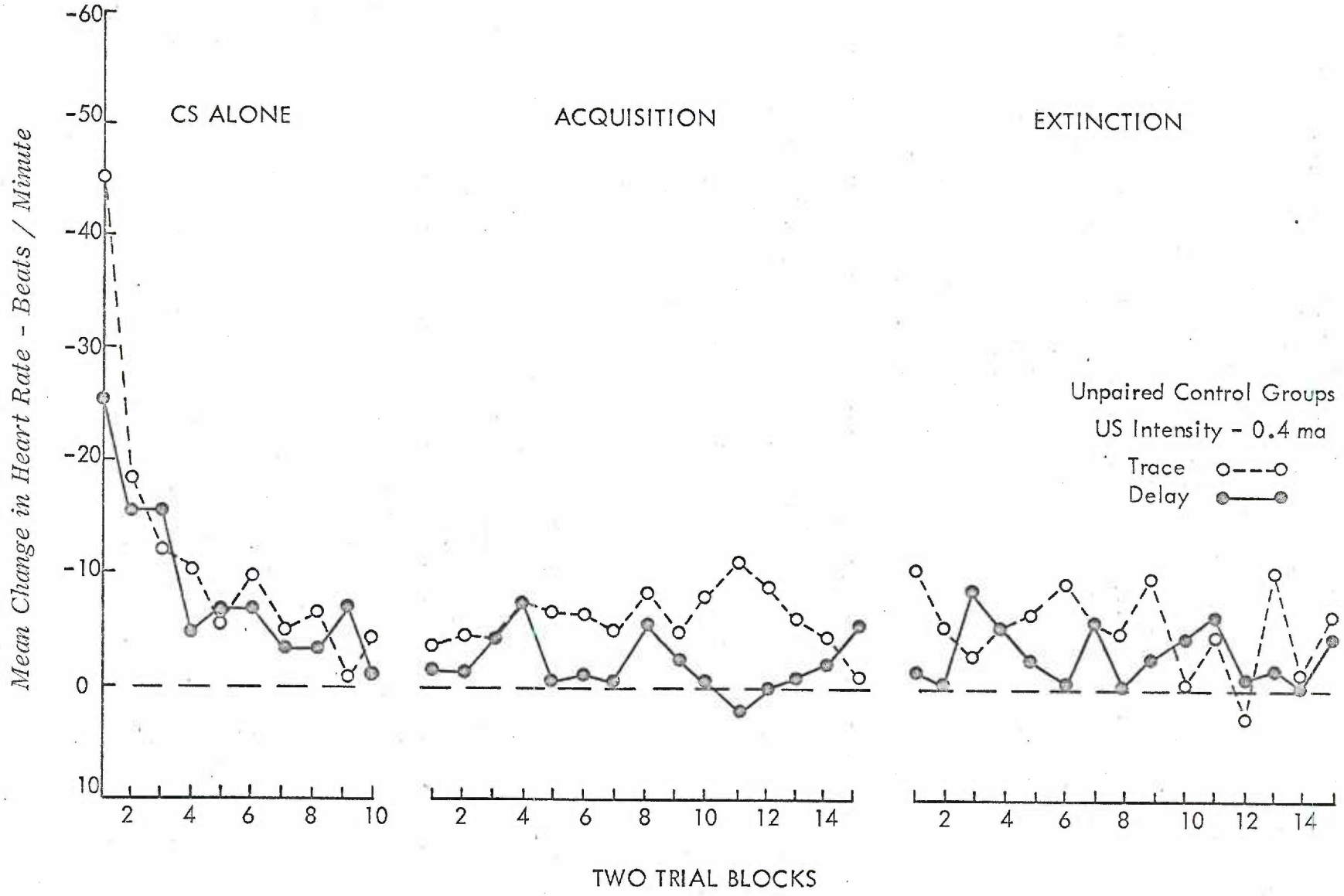
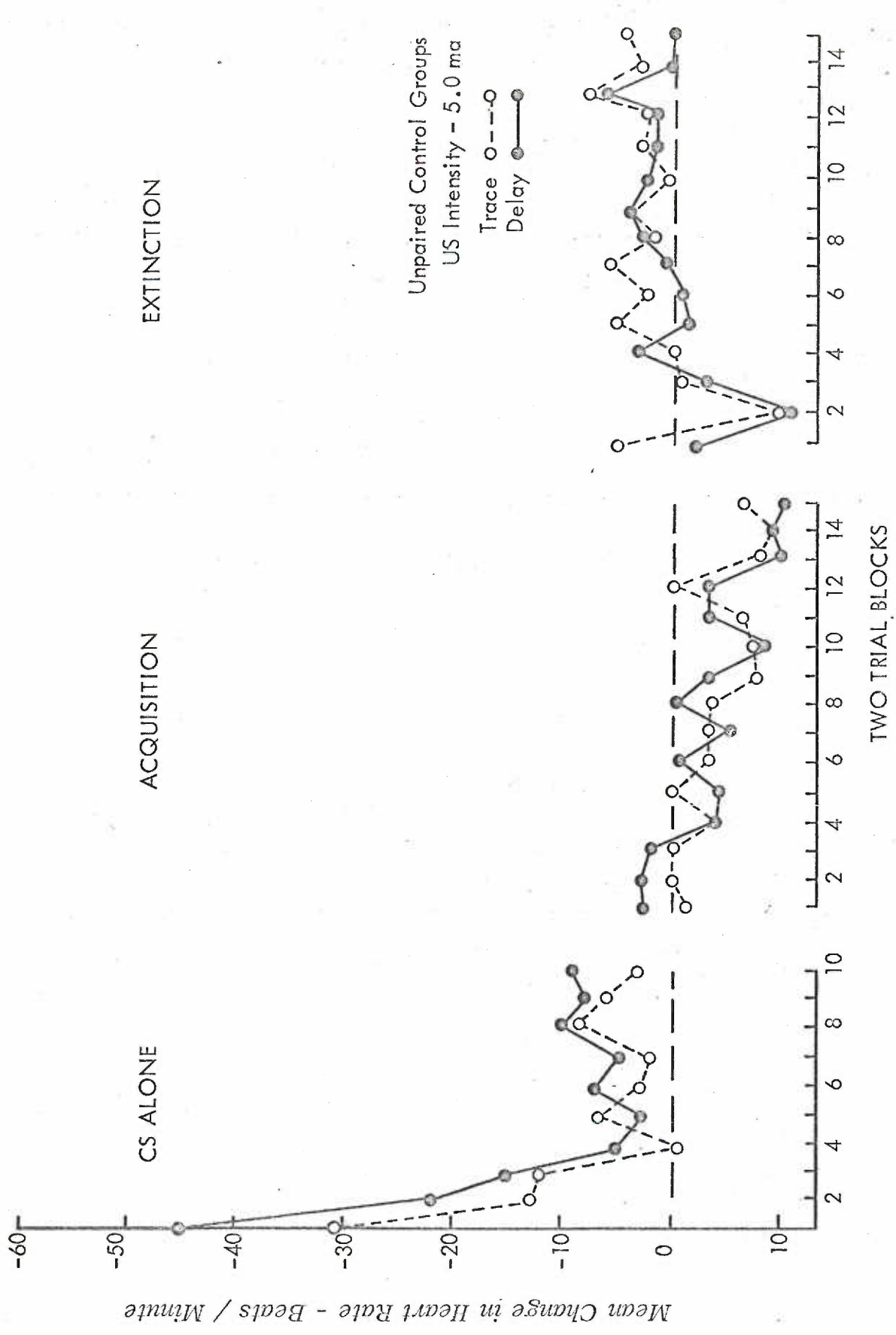

CS ALONE
ACQUISITION
EXTINCTION
Mean Change in Heart Rate - Beats / Minute
-60
-50
-40
-30
-20
-10
0
10
2
4
6
8
10
12
14
TWO TRIAL BLOCKS
Unpaired Control Groups
US Intensity - 0.4 ma
Trace
Delay

Figure 5a

Figure 5b


Mean Change in Heart Rate - Beats / Minute
-60
-50
-40
-30
-20
-10
0
10
CS ALONE
ACQUISITION
EXTINCTION
Unpaired Control Groups
US Intensity - 5.0 ma
Trace o--o
Delay
2
4
6
8
10
12
14
TWO TRIAL BLOCKS

is comparable to that of all the experimental groups. A 2x2x2 factorial analysis of variance on these and the matched experimental groups data revealed a significant effect of Trials (F=62.45, df=9/936, $p < .001$) and a significant interaction among Trials and Shock Intensity and Trace versus Delayed Conditioning and Experimental versus Control Treatments (F=2.81, df=9/936, $p < .01$). The interaction appears to be due, in part, to the various starting points of the groups in the CS alone phase of the experiment. No other factors were reliable.

The center portions of Figures 5a and 5b show the heart rate changes to the CS in the acquisition phase of the experiment. Figure 5a shows that the heart-rate responses of the 0.4 ma. control groups do not change systematically during acquisition. The 5.0 ma. control groups shown in Figure 5b, however, appear to show an accelerative response to the CS. This accelerative response to the CS was not observed in any other group in this study. An overall analysis of variance comparing the results of the four control groups with those of the matched experimental groups resulted in reliable effects of Shock Intensity (F=12.40, df=1/124, $p < .001$), Experimental versus Control Treatment (F=85.52, df=1/104, $p < .001$), and an interaction between Shock Intensity and Experimental versus Control Treatments (F=51.78, df=1/104, $p < .001$). The analysis also revealed a reliable effect of Trials (F=2.35, df=14/1456, $p < .01$), and significant interactions between Trials and Experimental versus Control Treatments (F=4.35, df=14/1456, $p < .001$), and Trials and Shock Intensity (F=1.90, df=14/1456, $p < .05$), and a reliable interaction among Trials and Experimental versus Control Treatments and Shock Intensity (F=5.32, df=14/1456, p - .001).

These data were collapsed across the trace and delayed factor in as much as it did not reach statistical significance and individual F tests were performed. The comparison between the 0.4 ma. experimental and control groups is significant ($F=4.71$, $df=1/108$, $p < .05$), inidicating a slight superiority of the experimental groups even at this low intensity of shock. The superiority of the experimental groups over the control groups was also significant at 5.0 ma. ($F=276.77$, $df=1/108$, $p < .001$). These results and the interaction in the analysis of variance reflect the marked superiority of the paired experimental groups over the unpaired control groups.

Mean difference scores of the control groups during extinction are presented on the right-hand panels of Figures 5a and 5b. There is little evidence of any systematic changes during extinction in these data. An analysis of variance on difference scores in extinction between the experimental and control groups resulted in significant effects of Shock Intensity ($F=48.35$, $df=1/104$, $p < .001$), Experimental versus Control Treatments ($F=76.59$, $df=1/104$, $p < .001$), and Trials ($F=2.02$, $df=14/1456$, $p < .05$), and significant interactions between Shock Intensity and Experimental versus Control Treatments ($F=67.66$, $df=1/104$, $p < .001$), Trials and Experimental versus Control Treatments ($F=2.75$, $df=14/1456$, $p < .001$), and Trials and Shock Intensity ($F=1.74$, $df=14/1456$, $p < .05$). These results are mainly attributable to the fact that the CR's of the 5.0 ma. experimental groups fail to show any decrement in extinction as compared to the 0.4 ma. experimental groups and the 0.4 and 5.0 ma. control groups, which had no CR's to-extinguish.

Unconditioned Response

The direction of the heart-rate response to shock (UR) was found to change across trials. To elucidate the nature of this change, mean UR difference scores were computed for the first four acquisition trials. This difference score for the experimental groups was obtained by subtracting the heart rate during the 6 sec. pre-CS period from the heart rate during the 6 sec. post-US period. Thus, a decrease in heart rate during the post-US period relative to the pre-CS period is reflected as a negative score. The same method of computing the UR difference score was used for the control groups except that a 6 sec. pre-US measure was substituted for the pre-CS measure. Table 2 presents the group mean UR difference scores for each of the first four acquisition trials for experimental and control groups. An inspection of individual row entries for the experimental groups shows that the heart-rate response generally changes from a deceleration to an acceleration across the four trials. Although not shown, the UR on trials 5-30 is similar to the UR on trial 4. An inspection of individual column entries for the experimental groups shows that the UR, whether decelerative or accelerative in nature, increases as a function of US intensity.

A factorial analysis of variance was performed on the UR data of the experimental groups. This test resulted in a reliable effect of Shock Intensity ($F=2.86$, $df=5/156$, $p < .05$), a reliable effect of Trials ($F=89.80$, $df=3/468$, $p < .001$), and a reliable interaction between Trials and Shock Intensity ($F=7.51$, $df=15/468$, $p < .001$). These results are consistant with the notion that the magnitude of the UR is determined

Table 2. Mean UR difference scores of the first four acquisition trials for experimental and control groups. For experimental groups the difference score is post-US heart rate minus pre-CS heart rate; for the control groups the difference score is post-US heart rate minus pre-US heart rate.

Table 2

			Trials in Acquisition				$\bar{X}_{trace}$	$\bar{X}_{delayed}$
			1	2	3	4		
Experimental Groups	0.4 ma	trace	-9.8	-21.0	-16.2	-5.2	-13.1	
		delayed	-9.0	4.4	7.4	1.8		1.2
	0.8	trace	-39.6	-12.2	-18.8	-21.4	-23.0	
		delayed	-37.0	-14.2	0.6	12.8		-9.5
	1.2	trace	-31.0	-19.4	1.2	12.0	-9.3	
		delayed	-77.2	-26.8	0.4	19.6		-21.0
	1.8	trace	-109.8	-24.4	4.6	9.0	-30.2	
		delayed	-70.0	-15.2	14.8	32.8		-9.4
	3.0	trace	-69.0	-3.6	25.6	83.8	9.2	
		delayed	-58.0	6.6	52.2	93.8		23.7
	5.0	trace	-40.0	27.2	40.8	59.4	21.9	
		delayed	-145.2	-26.2	37.2	63.7		-17.6
	$\bar{X}$	trace	-49.8	-8.9	6.2	22.9		
	$\bar{X}$	delayed	-66.0	-11.9	18.7	37.4		
Control Groups	0.4 ma	trace	4.2	22.4	15.6	12.8	13.8	
		delayed	-9.0	-11.6	-2.4	14.4		-2.2
	5.0	trace	-71.2	-27.4	28.6	52.8	-4.3	
		delayed	-99.0	-32.8	15.8	41.2		-18.7
	$\bar{X}$	trace	-33.5	-2.5	22.1	32.8		
	$\bar{X}$	delayed	-54.0	-22.2	6.7	27.8		

by the strength of the US and that the UR changes across trials. The analysis also resulted in a reliable interaction between Trials and Trace versus Delayed Conditioning (F=3.19, df=3/468, $p < .05$). This interaction is the result of the delayed groups showing a larger decelerative UR on trial 1 and attaining a greater accelerative UR on trial 4 than the trace groups.

Looking now at the UR data of the control groups in comparison to the experimental groups, it is evident that the same kinds of changes took place. The UR again generally changes from a deceleration to an acceleration across the four trials and the magnitude of the decelerative and accelerative components are greatest at the high shock level. A factorial analysis of variance on the control groups and their matched experimental groups resulted in a reliable effect of Trials (F=48.59, df=3/312, $p < .001$), and interactions between Trials and Shock Intensity (F=35.92, df3/312, $p < .001$) and Trials and Trace versus Delayed Conditioning (F=2.99, df=3/312, $p < .05$). These outcomes indicate that the control groups do not differ reliably from the experimental groups. The change in direction and magnitude of the UR is comparable for both experimental and control groups, suggesting that the presence of the CR has little effect on the nature of the UR.

Movement

Each S's median number of movements occurring in two-trial blocks was subjected to a CS period minus pre-CS period difference score analysis to get a measure of the relative amount of movement occurring during the pre-CS and CS periods. This computation resulted in group difference scores that do not differ from zero during the course of the acquisition.

Discussion

Before embarking on a detailed discussion of the CR data it is important to deal with the pre-CS changes in heart rate that were seen to occur in this experiment. It will be recalled that the pre-CS heart rate of the experimental Ss decreased substantially at the start of acquisition training. This decrease was followed on later trials by a gradual increase toward pre-acquisition levels. Since the CR scores were computed on the basis of these changing pre-CS values, it is clear that they may have been influenced by the different base levels. However, because the acquisition CR was a decrease in heart rate relative to the pre-CS level, we have a situation in which a pre-CS decrease in heart rate was followed by a further decrease in heart rate to the CS. Therefore, any bias that a decrease in pre-CS heart rate would impose on the CR is in the direction of reducing the magnitude of the CR rather than magnifying it. This follows, in part, from the law of initial values (Wilder, 1950; Wilder, 1957) which states that the magnitude of change of an ongoing autonomic nervous system response depends upon the initial level of that response. Applied to this situation a low basal heart rate would allow a decrease of lesser magnitude than a higher basal heart rate.

This same reasoning would also apply to the changes in pre-CS heart rate as a function of US intensity that were obtained. It was found that the higher the shock level the greater the decrease in pre-CS responding. It was also shown that CR magnitudes generally increased with increasing US intensity. Thus, if the pre-CS decreases affected the CR's at the

different US intensities, this effect would have been to minimize differences between groups.

Evidence that the CR was not an artifact of the pre-CS changes comes also from the observation that the pre-CS decrease was virtually eliminated in the latter part of acquisition while the CR remained stable. Furthermore, CR's in extinction were maintained even though pre-CS heart rate was not depressed.

The direction of the CR, for all Ss regardless of shock level, was a deceleration in heart rate. These results do not agree with those of Lang and Black (1963) who found in dogs that more decelerative responses occurred at low than at high shock levels. It should be pointed out, however, that there are obvious species differences that might account for the difference. The results also do not appear to support our initial hypothesis that the accelerative CR's reported in grid shocked rats (Black and Black, 1967; Fehr and Stern, 1965; McDonald, Stern, and Hahn, 1963) were due to the greater noxiousness of delivering the US to the densely innervated feet of the Ss. On the other hand, one could argue that 5.0 ma. chest shocks were not as noxious as 1.8 ma. foot shocks. The differences in direction of the CR as reported by Black and Black (1967), McDonald, Stern, and Hahn (1963), and Fehr and Stern (1965) as opposed to Holdstock and Schwartzbaum (1965) and Fitzgerald, Vardaris, and Brown (1966) are apparently due to other factors. The most likely explanation now seems to be the use of freely moving as opposed to restrained animals.

This study does not confirm the results of Black, Carlson, and Solomon (1962) who found that the direction of the heart-rate CR was

influenced by the use of trace or delayed procedures. They observed that trace conditioned dogs gave more decelerative CR's than delayed animals. It will be recalled that in the present study the CR for both the trace and delayed animals was a deceleration in heart rate. That these results do not agree with those of Black et al, may not be surprising when one considers that the latter experiment employed a different species of animal, dog, under the influence of the paralyzing agent, curare. It is possible, however, the the CS-US interval that we employed was not sufficiently long to produce this differential effect. It will be remembered that Black et al, observed their trace versus delayed differences at a CS-US interval of 10 sec.

The magnitude of the decelerative CR, in this experiment, was determined by the intensity of the US and by trace or delayed conditioning procedures. At low US intensities delayed was superior to trace conditioning, while at high US intensities there was no difference. These differential effects produced a significant interaction between US intensity and trace versus delayed procedures. The finding that the magnitude of the trace CR was seen to increase monotonically as a function of US intensity is in agreement with the results of many previous classical conditioning studies.

The effect of the US intensity variable on the CR performance of the delayed groups was quite different. This effect manifested itself as a decrease in CR magnitude with intense shocks. That the result was not an artifact of pre-CS levels has previously been established. An examination of the median CR values of the trace and delayed groups indicated that

they are sufficiently close to the mean values to eliminate the possibility of a skewed distribution affecting the statistical analyses.

Warstler and Ost (1965), in a classical conditioning study of the salivary response in dogs, also found that CR magnitude was diminshed at high US intensities. While offering no mechanisms for the effect the authors stated that perhaps had other studies used sufficiently intense US's they too would have obtained the inversion. The results of the delayed groups are consistent with the finding of Holdstock and Schwartzbaum (1965) that a more intense shock results in a CR of a lesser magnitude. The crucial factor in the present experiment is not so much that the inversion was observed for the delayed animals, but that it was not observed for the trace animals. It is perhaps significant in this regard that the other studies showing an inversion also employed a delayed conditioning procedure.

One explanation of the CR inversion at high US intensity levels is that the shocks interferred with CR formation through the mechanism of a powerful emotional reaction (Hilgard and Marquis, 1940). Unfortunately, this hypothesized mechanism would also predict a diminution in the CR's of trace animals at high shock levels.

Another explanation of the inversion is that because the CR magnitude of delayed Ss was substantial at low shock intensities, a ceiling was reached wherein the heart rate could decelerate no further. This is possible as there are known physiological limits that determine the maximum slowing of the heart rate (Ruch and Patton, 1965). However, if this ceiling effect were operative, one would expect to see a plateau in the magnitude of the CR rather than the actual inversion that was obtained.

A third possible explanation of the inversion can be offered in terms of the concept of inhibition of delay. That is, the CR might have been delayed until just prior to the US. This would result in a total difference score over the 6 sec. CS period that was actually less than would be obtained from animals not showing inhibition of delay. To account for the CR inversion using this mechanism it would be necessary to demonstrate that the delayed groups exhibited more inhibition of delay at high shock levels than at low shock levels.

The results of this experiment showing that the magnitude of the delayed CR was greater than that of the trace CR are consistent with the findings of other studies (Church and Black, 1958; Ellison, 1964; Pavlov, 1927). A comparison of trace and delayed groups revealed that at no point was the magnitude of the trace CR reliably greater than that of the delayed CR. This relative inefficiency of trace procedures is commonly attributed to the decay of the stimulus trace during the interval of time between the offset of the CS and the onset of the US (Pavlov, 1927; Kamin, 1961). This notion is supported by the finding that a trace procedure becomes progressively less effective in producing a CR relative to a delayed procedure as the CS-US interval is lengthened (Church and Black, 1958; Ellison, 1964). The proposed mechanisms for this effect include the suggestion of Pavlov (1927) that the stimulus trace in the central nervous system weakens as a function of time after CS offset. The present results showing that delayed conditioning was superior to trace conditioning could be accounted for in terms of the stimulus trace hypothesis.

An alternative to the stimulus trace hypothesis is that responses develop during the trace interval that are incompatible and interfere

with the formation of the CR (Ellison, 1964). It is assumed that these incompatible responses do not occur in delayed conditioning because of the presence of the CS. The incompatible response hypothesis is not supported by the present results in that gross body movements were not seen to occur in the trace interval. However, this finding does not eliminate the presence of other mediating responses that were not measured. For example, movements too discreet to be recorded by the present apparatus could have occurred and affected the formation of the CR. Unmeasured central mediating responses could also fit the incompatible response hypothesis.

The extinction data indicated that the higher the acquisition CR the greater the initial level of responding in extinction and the greater the response decrement over extinction trials. The only exception to this was in the 3.0 and 5.0 ma. shock groups, which showed no response decrement during extinction. This result could be due to the intense shock producing a powerful emotional reaction in the Ss, such that they tended to perseverate learned behavior. The observation that the pre-CS heart rate did not decrease in extinction for these groups as compared with all others also suggests the presence of a high level of arousal.

At this point it would seem appropriate to provide more detailed comments on the pre-CS heart rate changes that were found in acquisition. The one previous study that analyzed pre-CS scores (Fitzgerald, Vardaris, and Brown, 1966) did not find a decrease in pre-CS heart rate on the first day of conditioning. The 100% group in the Fitzgerald et al, study was almost identical to the 1.8 ma. delayed group in the present study, yet the pre-CS values did not show the decline. The pre-CS decrease was,

however, seen on the second day of conditioning. Referring to the lack of a pre-CS decrease on the first day, one main procedural difference was that the ITI was 10 min. rather than 3 min. as used in the present study. It will be shown below how this variable may have affected the results.

In the present study the control groups did not show pre-CS decreases during acquisition. To measure temporally comparable post-shock heart rates for the control groups as compared with the experimental groups, pre-US heart-rate scores were substituted for pre-CS measures. Figures 6a and 6b show these pre-US measures plotted for the 0.4 and 5 ma. control groups. These figures show that a decrease in heart rate occurred during the pre-US period for the high shock control groups but not for the low shock groups.

The observed decreases for the high shock control groups suggest that the temporal relationships of the shocks may have played a significant role in determining pre-CS heart rate of the experimental groups. To test for this possibility, four naive rats were placed in the experimental situation and following a one-half hour period of adaptation, given 25 trials of a 1.8 ma. shock at an ITI of 3 min. No other stimuli were present. Heart-rate samples were taken every 20 sec. during the ITI to determine the nature of the UR to shock. The UR to the first shock was a heart-rate deceleration relative to the baseline heart rate taken prior to the first shock. The UR to the second through twenty-fifth shock was an initial acceleration in heart rate followed by a deceleration. This deceleration was maximal on trials 6--11, declining thereafter. This finding corresponds with that of the present investigation where it was

Figures 6a and 6b. Mean pre-US heart rate in beats/minute for each control group as a function of two-trial blocks in acquisition. The two curves in each figure represent the trace and delayed groups at the indicated US intensity.

Figure 6a

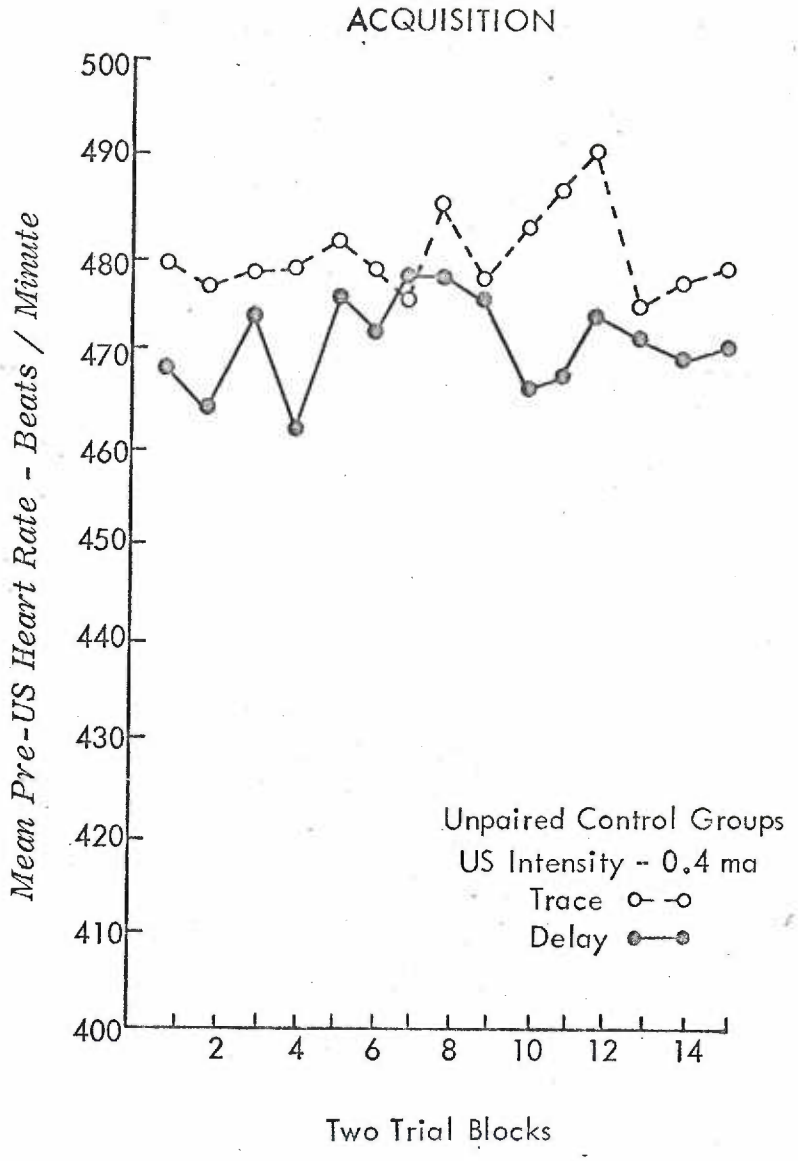
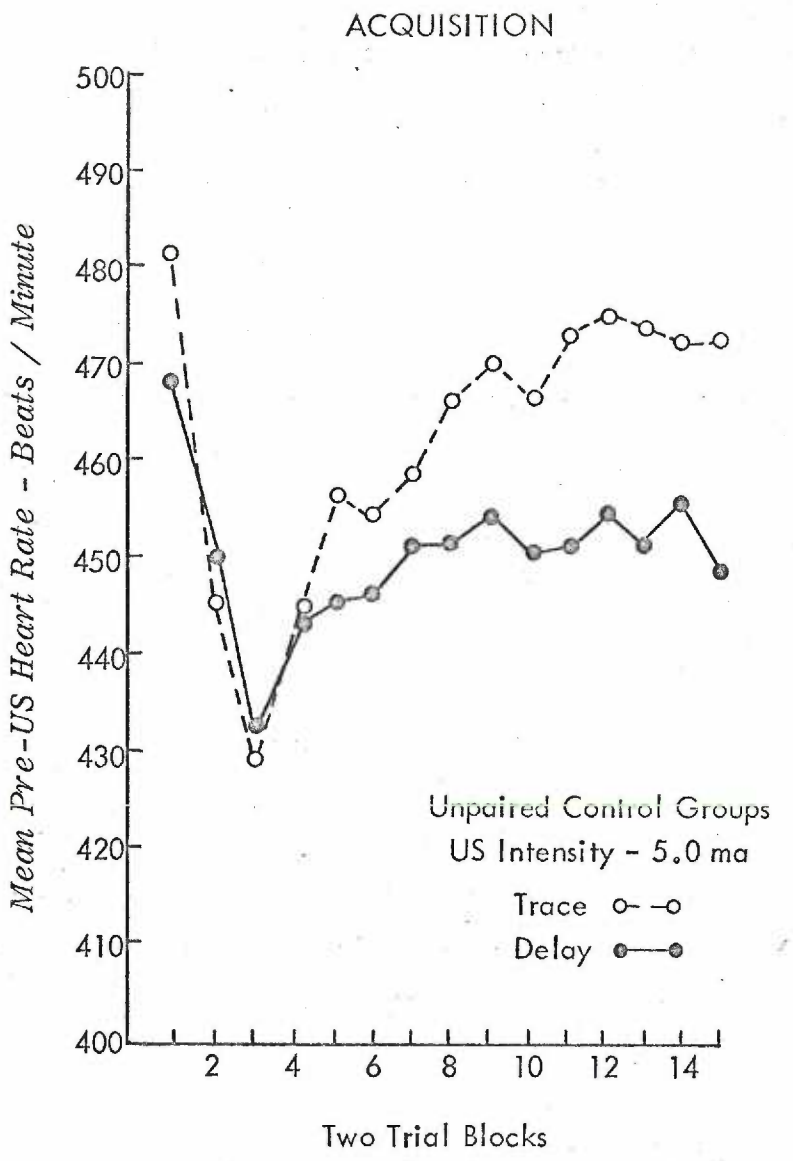

ACQUISITION
Mean Pre-US Heart Rate - Beats / Minute
500
490
480
470
460
450
440
430
420
410
400
2
4
6
8
10
12
14
Two Trial Blocks
Unpaired Control Groups
US Intensity - 0.4 ma
Trace
Delay

Figure 6b


ACQUISITION
Mean Pre-US Heart Rate - Beats / Minute
500
490
480
470
460
450
440
430
420
410
400
2
4
6
8
10
12
14
Two Trial Blocks
Unpaired Control Groups
US Intensity - 5.0 ma
Trace
Delay

observed that the maximum pre-CS acceleration occurred on the third two-trial block. Therefore, the decrease in heart rate as measured in the pre-CS period was actually a part of the UR to shock. The point in time at which the response crossed over the baseline from an acceleration to a deceleration was from 80-100 seconds after US onset. This explains why a decrease in heart rate was not seen in the pre-CS scores of the high shock control groups. In this case the CS followed by the US a mean of 90 sec., an interval of time that would effectively sample the UR as it was crossing the baseline from an acceleration to a deceleration. Referring to the Fitzgerald et al, study, the effect was not obtained on the first day presumably because the effects of the US normally do not last for 10 minutes, which was the ITI utilized in that study. The pre-CS decreases on the second day of the Fitzgerald et al, study are difficult to account for unless one hypothesizes a strong emotional response by the Ss that effectively changed the time course of the UR to shock.

The present experiment demonstrated that the UR on initial presentations of the US was a deceleration whereas on the later trials it was an acceleration. Since the UR was the same for the control groups as it was for the experimental groups, it can be concluded that the CR had little effect on the UR. However, the presence of the CS did appear to affect the UR. This is indicated by the fact that there was a significant interaction between trials and trace versus delayed conditioning on UR scores. This interaction indicated that the UR was greater for the delayed groups than for the trace groups.

One possible explanation for the decelerative UR on early trials lies in the novelty of the US on its initial presentations. Whereas the

original response to the CS was a deceleration it may be that any novel stimulus, even a noxious US that would normally produce a heart rate acceleration, could result in a heart-rate deceleration in rats. After several presentations of the US it would presumably no longer be novel and the accelerative response to the painful qualities of the US would remain.

The group mean heart-rate levels in the adaptation phase of the experiment did not differ from one another and there was no decrease in heart rate over the four sampling periods. This finding is in direct contradiction to studies showing a decrease in basal heart rate as a function of time in a new environment (Black, Fowler, and Kimbrell, 1964). In the Black et al, study, the heart rate of freely moving rats was seen to drop from about 450 beats/min. to about 370 beats/min. after 20 min. In the present study the mean heart rate remained at about 480 beats/min. during the course of the 20 min. period. The higher initial level and the fact that the heart rate did not decline with time may be due to the procedure of restraining the animals in the present study.

The interpolated US control groups differed reliably from their matched experimental groups. This finding indicates that pseudo-conditioning and sensitization effects played a minor, if any, role in the present study. Moreover, the direction of the heart-rate response in acquisition for the 5.0 ma. control Ss was opposite to that of the experimental groups. The heart rate of the 5.0 ma. experimental groups showed a dramatic deceleration while the heart rate of the 5.0 ma. control groups showed a slight acceleration. That this effect was not seen in the 0.4 ma. control groups is undoubtedly due to the ineffectiveness of

the 0.4 shock. This intensity of shock was, however, sufficient to support the development of a reliable CR in the experimental group. The accelerative heart rate reaction seen in the 5.0 ma. control groups is an interesting result that bears on recent statements by Rescorla (1967) and Prokasy (1965). Their investigations maintain that the CS in an interpolated US control procedure can effectively signal a "safe period" to the S. The response to a stimulus signalling a "safe period" would presumably be different from the response to a stimulus signalling an upcoming aversive event. The accelerative response of the control groups could then be viewed as reflecting the operation of this mechanism.

The implications of the results of the present study will now be examined in relation to several major theories of conditioning.

Traditional stimulus substitution theory dates back to the works of Pavlov (1927). In its simplest conception stimulus substitution theory maintains that the pairings of the CS and US permits the CS to be substituted for the US. According to this theory the CR and UR should be similar. The contiguity theory of Guthrie (1952) assumes that the mere occurrence of a stimulus and a response in contiguity results in a complete association between them. According to this theory, an association is developed between the CS and UR and thus the CR should be similar to the UR. Both formulations have difficulty accounting for the present results as the CR and UR were in opposite directions. Evidence contrary to these theories has been provided by several other studies (Fitzgerald, Vardaris, and Brown, 1966; Notterman, Schoenfeld, and Bersh, 1952; Zeaman, Deane, and Wegner, 1954).

The most detailed formulations of drive reduction theory have been put forth by Hull (1952) and Spence (1956). The core of the theoretical system is that whenever a response is contiguous to a stimulus, and this stimulus-response contiguity occurs near in time to the reduction of a drive or a drive-stimulus, the stimulus-response association is strengthened. Reinforcement is thus defined in terms of drive reduction. This hypothesis suggests that the CR should resemble the UR at the time of US termination. Drive reduction theory also maintains that the termination of an intense US is more reinforcing than the termination of a weak US and that, therefore, intense US's should produce better conditioning.

In the present experiment the UR was accelerative and the CR was decelerative. It is clear that these data do not agree well with the hypothesis that the CR resembles the UR at the time of US termination. However, it must be remembered that the heart rate at the time of US termination could not be measured due to technical difficulties, and that the heart rate as measured 1 sec. later may have been quite unlike the heart rate at US termination. The results of the trace groups accord with the prediction of a larger CR with a more intense US, whereas the data of the delayed groups, showing an inversion in the CR magnitude function, do not completely fit the drive reduction model. The inadequacy of the drive reduction formulation has been referred to in other studies (Zeaman and Wegner, 1954; Zeaman and Wegner, 1958).

Mediation theory maintains that the pairing of the CS and the US results in the association of the CS with a mediating state or response, which in turn affects some aspect of behavior. Mediating states have been hypothesized to include fear, anxiety, and expectancy; while medi-

ating responses would include autonomic and skeletal activity. Mediation theory is an extension of Hullian learning theory, yet the emphasis upon the mediating process and the resultant response-produced stimulation bring this view close to Guthrie's theory. The influence of Tolman (1932) can also be seen if one views mediation theory as a more explicit formulation of "sign-significant—expectations". Bersh, Notterman, and Schoenfeld (1957) utilize mediation theory to explain the results of their series of experiments on the human heart-rate response. The decelerative CR that they observed was explained in terms of mediated anxiety, a state which results in cardiac deceleration. Deane and Zeaman (1958) postulate a dual mediation theory to account for the accelerative and decelerative CR's they obtained in human heart-rate study. In their conception, when warnings of approaching aversive stimuli are primarily response-produced (verbal) cues, the result is anxiety, a state with a diffuse time course and a physiological correlate of heart-rate acceleration. When the warning cues are external, the result is fear, a state with a more discreet temporal property, and a physiological correlate of cardiac deceleration. Steward (1962) modified the dual mediation theory of Deane and Zeaman on the basis of obtaining a cardiac deceleration to a US which was a "pleasant sound". Steward assigned "attention" or "alertness" to the external cues rather than fear since pleasant sounds are not usually thought of as being fear producing. The interpretation of cardiac deceleration as mediated by fear was further weakened by Smith (1963). He found no decelerative response in a controlled respiration study similar to a study that obtained cardiac deceleration without respiratory controls

(Deane and Zeaman, 1958). He has, in effect, proposed a respiratory mediation theory to account for cardiac deceleration .

In the present study there was no evidence to support the view that the CR was mediated by skeletal movements, as movements were very infrequent. It is possible, however, that had more sensitive recording devices been used more skeletal movements would have been recorded. While respiratory activity was not quantified, it was observed that there were no obvious respiratory irregularities that might be conceived of as mediating the CR.

A recent interpretation of classical conditioning deserves mention here. This is the notion of the instrumental consequences of the CR as having adaptive value for the S. Kimmel (1966) has attempted to explain the effect of inhibition of delay as due to differential reinforcement of late responses. In other words, the CR in an aversive classical conditioning situation somehow reduces the noxiousness of the subsequent US. Another view along these same lines is the punishment model of Fitzgerald (1966). This notion, which was developed to offer a mechanism for the partial reinforcement effect, can also be applied to a 100% reinforcement schedule. According to this model, competing responses, which are assumed to be responsible for the major loss in CR strength during extinction, occur during acquisition in partial reinforcement as a result of non-reinforced trials. It was argued that when competing responses are present on reinforced trials during acquisition, they are punished and that this punishment reduces the probability of their occurrence during extinction. It follows from this view, that the CR is somehow instru-

mental in reducing the noxiousness of the US since if it were not, it would tend to be eliminated through the mechanism of punishment.

It is conceivable that the CR's seen in the present study reflected the operation of an instrumental response the result of which was to render the US less aversive. That the CR was larger at more intense shock levels is consistent with the hypothesis that a highly aversive event requires a more vigorous instrumental response to neutralize it.

Summary

An experiment was conducted to determine the effects of trace versus delayed conditioning of heart rate in restrained rats as a function of US intensity. Six intensities of 1 sec. shock (0.4, 0.8, 1.2, 1.8, 3.0, and 5.0 ma.) were factorially combined with trace and delayed conditioning procedures. The CS was a 1000Hz tone of 1 and 7 seconds duration for the trace and delayed groups, respectively. The CS-US interval was 6 sec. All Ss received a 30 min. adaptation period, 20 CS-alone trials, 30 acquisition trials, and 30 extinction trials. The ITI during acquisition and extinction was variable with a mean of 3 min. An interpolated US conditioning control was employed. The index of conditioning was a CS minus pre-CS difference score.

The principal findings were:

1) The original response to the CS for all Ss was a pronounced initial heart-rate deceleration that habituated to near zero after 20 CS alone trials.
2) The CR was also a heart-rate deceleration for both trace and delayed procedures regardless of shock intensity.
3) The UR was a deceleration in heart rate on acquisition trials 1 and 2 and an acceleration in heart rate on trials 3 through 30.
4) The magnitude of the CR increased with increasing US intensity for the trace groups. The function describing the CR magnitude of the delayed groups exhibited an inversion at high shock levels.
5) Extinction performance was directly related to the magnitude of the acquisition CR, except for the 3.0 and 5.0 ma. experimental groups

that showed no response decrement during extinction.

6) Experimental groups showed a large decrease in pre-CS heart rate that increased in magnitude as US intensity increased. There were no differences between trace and delayed groups pre-CS heart rates.

It was concluded that neither shock intensity nor trace versus delayed procedures have an effect on the direction of the cardiac CR of rats in this situation. The non-linearity of the delayed groups CR data is inconsistent with the general effects of increasing US intensity on the magnitude of the CR in other situations. The linearity of the delayed groups CR's as a function of US intensity agrees with the results of many previous investigations. The pre-CS changes that occurred were hypothesized to be a component of the UR to shock; a pilot study lent support to this hypothesis. As the CR was a deceleration in heart rate and the UR was an acceleration in heart rate it was concluded that stimulus substitution theory could not adequately account for the results of this experiment. Mediation theory cannot account for the present results in that no systematic changes in gross movement were demonstrated, although other mediating responses could have occurred that were not measured. The results could be interpreted in terms of an instrumental conditioning theory.

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