OPERANT CONDITIONING OF HEART RATE IN THE PARALYZED RAT

OPERANT CONDITIONING OF HEART RATE IN THE PARALYZED RAT

by

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SCOPE AND CONTENTS: Punishment training was carried out in the three experiments in an attempt to operantly condition heart rate in the paralyzed rat. A fourth experiment compared the effect of two paralyzing agents, d-tubocurarine and succinylcholine, on cardiovascular and electrodermal responding in an attempt to improve the paralyzed preparation. Reliable bidirectional differences in heart rate were produced in two of the learning experiments, one using d-tubocurarine and the other employing succinylcholine as the paralyzing agent. In a third experiment, rats paralyzed with succinylcholine failed to display bidirectional differences in heart rate by the end of conditioning. The bidirectional changes that were produced were specific to the cardiac response. Skin-potential responding was not systematically affected by punishment training. Alternative interpretations other than operant conditioning are considered and the current status of operant conditioning of autonomic responses under curare is evaluated.

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CHAPTER ONE

INTRODUCTION

Historically, the autonomic nervous system (ANS) was viewed as a vegetative involuntary system consisting of the nerves, ganglia and plexuses, that innervate the heart, smooth muscles, glands, viscera and blood vessels of the body. Consistent with this viewpoint was the notion that responses of the ANS were not susceptible to voluntary control or to operant conditioning, processes which many believed to apply only to the skeletal-muscular system. Both Mowrer (1947) and Skinner (1953) believed that responses of the somatic nervous system could be operantly conditioned but that responses of the autonomic nervous system could be modified only by classical conditioning procedures. This differentiation between somatic and autonomic responses was accepted for many years (Kimble, 1961).

During the past ten years this interpretation has been challenged by two main lines of evidence. The first comes from a large literature demonstrating operant or voluntary control of heart rate (HR) and other ANS responses in human subjects.

In an early experiment in this area, Brener and Hothersall (1966) found that human subjects were able to control their heart rates when provided with feedback for cardiac responding. They demonstrated that subjects were able to raise or lower their heart rates during the presentation of stimuli which indicated that short inter-beat intervals (IBIs) or long IBIs would be followed by reward. Furthermore, the difference between the HR accelerations

and decelerations during raise and lower stimuli increased gradually throughout training and was highly reliable by the end of the session. Subsequent research by Brener et al. (1969) demonstrated that the amount of heart-rate control exhibited by subjects was a direct function of the percentage of trials on which feedback was given. The ability of human subjects to alter their heart rate when given feedback for performance has been reported by many other investigators as well, including Shearn (1962), Engel and Chism (1967) and more recently by Shapiro, Tursky and Schwartz (1970). Although one can question the conclusion that the changes reported in these studies were produced by the operant contingency, there can be little doubt that the subjects were able to comply with instructions requiring them to voluntarily raise or lower their heart rates.

An interpretative problem was raised, however, by another finding of this research. Although it was clear the voluntary control had been established, it was also apparent that this control was not specific to the cardiovascular system. Brener and Hothersall (1966) suggested that the heart-rate control was mediated by changes in respiration as gross differences in respiratory pattern were observed between acceleration and deceleration trials. The finding that heart-rate changes were accompanied by changes in respiration pattern or movement was observed by other investigators as well (Brener, Kleinman & Goesling, 1969, Engel & Chism 1967). This raised the possibility that the ANS had not been manipulated directly at all, but that the skeletal motor system was influencing the heart-rate response.

The second line of evidence originated as an attempt to rule out overt movement correlates of the heart-rate changes. This was accomplished by paralyzing the skeletal muscular system of rats with curariform drugs. The

majority of this work emanated from the laboratory of Miller and DiCara and received considerable attention as it appeared to demonstrate the independence of somatomotor and cardiovascular control.

The first experiment of this series was conducted by Trowill (1967). Using electrical stimulation of the brain (ESB) as a reinforcer, he trained one group of rats to increase and another group to decrease their heart rates while paralyzed with curare. At the end of training, a small but statistically reliable bidirectional difference in heart rate was produced.

Miller and DiCara (1967) attempted to amplify the small bidirectional difference reported by Trowill through the use of a shaping procedure. As this technique was applied to a number of autonomic responses, it will be presented in some detail.

All rats were first trained for three days to bar press for brain stimulation on an FI schedule. The purpose of this training was to ensure that the ESB was rewarding. The following day, heart-rate training began. Rats were paralyzed with curare and respirated with a positive pressure respirator (E & M V5KG) set at 70 cycles per minute, with an inspiration - expiration (I/E) ratio of 1:1 and a peak inspiratory pressure of 20 cms of water. After a 30 minute adaptation period, a criterion heart rate was chosen which could be achieved on the average of once every 5 seconds. Then, the reward circuit and discriminative stimuli (which were the same as those used in bar-press training) were turned on. As soon as the animal's heart rate surpassed criterion, reward was administered and the discriminative stimuli were turned off. A 20 second time-out followed, after which the reward circuits and discriminative stimuli were turned on again. These events constituted a trial. The criterion heart rate was not changed until the rat

required only half as long to meet criterion as it had initially. Then a new criterion was established, approximately 2% different from the previous one. On the other hand, criterion was relaxed if for a period of several minutes the rat was unable to achieve the required rate in 10 seconds. Heart-rate training was carried out for 90 minutes and no extinction period was employed.

A large (20%) bidirectional difference in heart rate was produced with this shaping procedure. Following the initial study, Miller and DiCara carried out a series of experiments to examine the properties of autonomic operants. They determined that this shaping procedure was successful with both punishment (DiCara & Miller, 1968a) and reward (Miller and DiCara, 1967). Furthermore, as with skeletal operants, learned changes were acquired gradually and extinguished when reinforcement was discontinued (DiCara & Miller 1968). The shaping procedure was applied successfully to many other autonomic systems as well, including blood pressure (DiCara & Miller, 1968c), urine formation (Miller & DiCara, 1968), vasodilation and vasoconstriction (DiCara & Miller, 1968b), intestinal motility (Miller & Banuazizi, 1968) and differential vasomotor responses in the two ears (DiCara & Miller, 1968d). Of additional interest was the fact that the learning that was observed was specific to the response that was reinforced. For example, reinforcement for fast or slow heart rates led to bidirectional differences on this measure, but did not affect intestinal contractions. Reinforcement for intestinal contractions, on the other hand, produced bidirectional differences in intestinal motility that were independent of heart rate (Miller & Banuazizi, 1968).

An important feature of this research was that mediation of the heart-rate changes by proprioceptive feedback from overt movement was ruled out as

curare was employed to paralyze the animals. But does paralysis of the skeletal musculature by curare exclude the possibility that central movement processes may be influencing heart rate? An experiment by Goesling and Brener (1972) indicated that the answer to this question was no. These investigators first trained separate groups of rats either to move (run in a running wheel) or to hold still in order to avoid electric shock. After the discriminative movement and correlated heart-rate responses had been established in the normal state, the rats were paralyzed and then trained to raise or lower their heart rates in order to avoid electric shock. Goesling and Brener found that the mobility-immobility pretraining administered in the normal state influenced heart rate under curariform paralysis, even though the skeletal musculature was paralyzed. This work, together with a study reported by Black (1967) called into question the adequacy of curarization as a control for movement processes, as it indicated that these processes still influenced heart rate in the paralyzed rat.

In response to this issue, DiCara & Miller (1969a) employed transfer tests to assess the role of movement processes in the learning of the heart-rate responses under curare. Rats were first trained to raise or lower their heart rates under curare and then were permitted to recover from paralysis. Two weeks later, they were tested for transfer of heart-rate changes to the normal state. Immediately following the transfer trials, additional heart-rate training was administered in the normal state. The results of heart-rate conditioning under curare are presented in Panel 1A of Figure 1. Inspection of Panel 1A reveals that statistically reliable bidirectional differences in heart rate were trained under curare. Perusal of Panel 1B indicates that the groups also differed reliably in the direction of training by the end of

FIGURE 1 - From DiCara and Miller (1969)

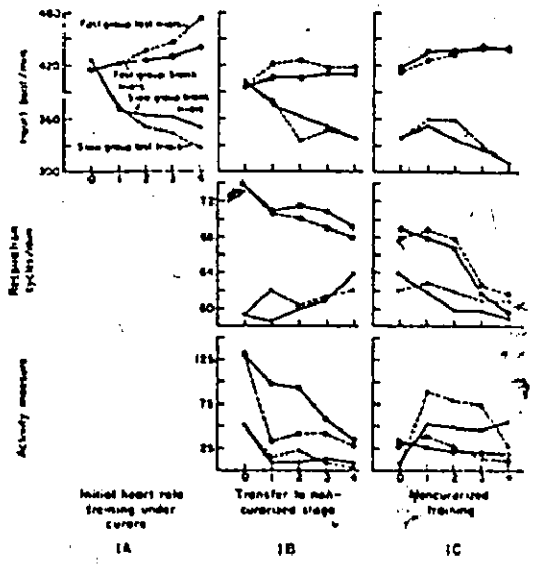


FIG. 1. Initial heart-rate learning under curare and changes in heart rate, respiration rate, and activity during test for transfer of heart-rate learning to the noncurarized state and subsequent heart-rate training in the noncurarized state. (Measures were obtained at quarterly intervals during training from individual blank and test trials.)

the transfer test. The additional training the animals received after the transfer test further amplified the bidirectional heart-rate difference (Panel 1C). As the transfer test and additional training were carried out in the normal state, measures of activity and respiration were available. Figure 1 indicates that although the bidirectional groups differed on both these measures at the beginning of the transfer test (Panel 1B), there was no difference between the groups in either respiration or activity by the end of retraining (Panel 1C). The relationship between bidirectional differences in heart rate and somatic variables at the beginning and end of training in the normal state, and changes in these variables over the course of testing, were not compatible with the view that the heart-rate changes were secondary to movement or respiration responses. Based upon this and a similar study (DiCara & Miller, 1969b), Miller & DiCara concluded that changes in heart rate were produced by an effect of conditioning that was specific to cardiac control fibers.

In 1969, Neal Miller published a review article on the learning of visceral and glandular responses (Miller, 1969). This represented the culmination of work from the Miller and DiCara laboratory and will probably be regarded as the high point for operant conditioning of autonomic responses under curare. Miller's thesis was that the ANS was not an inferior system as was once believed. Supported by data from his own laboratory, he described great plasticity in the ANS, and indicated that the learning of autonomic responses was even better under curare than in the normal state. Furthermore, he stressed that specificity within the ANS could be conditioned and reflected upon the future uses of operant autonomic conditioning in psychosomatic medicine.

However, soon after the publication of this paper, researchers in other laboratories reported that they were unable to produce such large changes in heart rate (Hothersall & Brener, 1969; Brener, 1970). Furthermore, the magnitude of the heart-rate changes from the Miller laboratory was also decreasing (DiCara, Braun & Pappas, 1970). In 1972 Miller himself (Miller, 1972) first reported difficulty in repeating the earlier heart-rate conditioning experiments. For these reasons, instead of pursuing work on the mechanisms of control, attention shifted to the more basic question of the replicability of the earlier Miller and DiCara work.

The research reported in this thesis was begun late in 1971 and it became immediately apparent that control of heart rate in the paralyzed rat was difficult to establish. The organization of the research to be reported is as follows. Chapter two presents a first attempt to operantly condition heart rate in the paralyzed rat. The next chapter compares cardiovascular and electrodermal functioning under two paralyzing agents in an attempt to improve the curarized preparation. Chapters four and five report two additional attempts to operantly condition heart rate. These experiments employ a preparation that takes into account the research from this and other laboratories on the problems of the curarized subject. The final chapter reconsiders the problems of operant heart-rate conditioning in light of the recent failures to replicate and examines the current status of heart-rate conditioning under curare.

CHAPTER TWO

EXPERIMENT 1

The experiment reported in this chapter represents an attempt to operantly condition heart rate in the paralyzed rat. The procedure employed is a modification of the variable criterion schedule of Fields (1970a,b).

Method

Subjects

The subjects were 34, male Wistar albino rats and 2 male hooded rats ranging in weight from 370-480 gms. Twelve animals were discarded or died during adaptation. Two of these were discarded because of equipment problems, seven displayed heart rates that never stabilized and three died during adaptation. Each subject was food and water deprived for approximately 18 hours prior to the experiment.

Procedure

All animals were injected intraperitoneally (i.p.) with 3.6 mg/kg of d-tubocurarine chloride (Tubarine, Burroughs Wellcome & Co., solution containing 3.0 mg/cc). Subjects were fitted to a face mask cut from a rubber balloon as soon as they experienced difficulty in breathing. The mask was connected to the tubing of an E & M respirator (model V5KG) via a rubber stopper with a hole drilled through the middle. Respiration settings of 70 breaths per minute with an I/E ratio of 1:1 were employed as it was believed at this time that these settings would maintain a rat in a healthy condition

for several hours. The pressure setting of the respirator was maintained at 20 cms of water, with a slightly higher setting for heavier animals and a lower setting for lighter animals, as recommended by DiCara (1970).

Curare was infused into the left thigh muscle at the rate of 0.8 mg/hr for the duration of the session, using a Harvard infusion pump (model #975). As most animals in this experiment weighed approximately 400 gms, this rate provided a nominal infusion of 2.0 mg/kg/hr.

Shock electrodes were positioned approximately 40 mm apart on the middle third of the rat's tail. The electrodes were constructed of pieces of zinc measuring 45 mm long and 5 mm wide and were sanded and coated with Beckman electrode paste prior to each use. Shock was a capacitor discharge (100 uf at 150 volts) through a series resistance totalling 375 k-ohms. This configuration, along with digital timing circuits, provided a 0.5 ma shock of 27 msecs duration that was delivered 1.2 msecs after the completion of the R-wave.

The animal rested on a lucite platform that was tilted 7° head-down and enclosed in an acoustically insulated chamber. Temperature in the experimental room was maintained at 78°F. Recording took place in an adjacent room.

Electrophysiological Recording

Electrophysiological data were recorded on a six channel Beckman oscillograph (Biomedical type R) operating at a chart speed of 2.5 mm/sec.

Beckman Ag/AgCl miniature skin electrodes were used for recording skin potential (SP). Active and reference electrodes were shorted together in a 0.1M NaCl solution and were selected prior to each application to ensure standing potentials of less than 1 mv. The SP electrodes were attached to

rubber grommets by electrode collars, moistened with Beckman electrode paste, and then filled with a unibase paste prepared to a molarity of 0.1M with respect to NaCl (Lykken, 1967). The active electrodes were secured over the foot pads on the rear feet. The upper thighs were shaved, drilled lightly, and anethesized locally with Xylocaine ointment before the reference electrodes were secured. SP responses from both rear feet were recorded at a sensitivity of 5 mv/cm.

Subcutaneous heart-rate electrodes, made from stainless steel hypodermic needles (#27), were coated with Xylocaine and inserted into an area ventral to the right forelimb and dorsal to the left hindlimb. The EKG was recorded directly on one channel of the polygraph. The power amplifier of this channel triggered a logic circuit (BRS digibits) that measured the duration of the RR interval to within $\pm$ 0.2 msecs. The EKG and the state of the logic circuit were monitored by separate beams of a dual-beam oscilloscope (Tektronix 502A) on a beat by beat basis, to ensure that the circuit was triggering on the R-wave and not some other component of the EKG. Heart rate was also recorded on another channel using an expanded range cardiotachometer (Beckman 9857B).

Conditioning Procedure

After application of the appropriate electrodes and transfer into the sound-attenuated chamber, the rat was left undisturbed for 60 minutes while electrophysiolocial recording was carried out. At the end of this adaptation period, the PR interval and the amplitude of the R-wave were calculated from the oscilloscope and recorded. This was followed by a 15 minute operant level period during which the animal remained undisturbed.

Conditioning was then carried out for 90 minutes in the following way. For each animal a distribution of 200 consecutive RR intervals was displayed on a bank of 40 counters. Distributions from a typical animal are presented

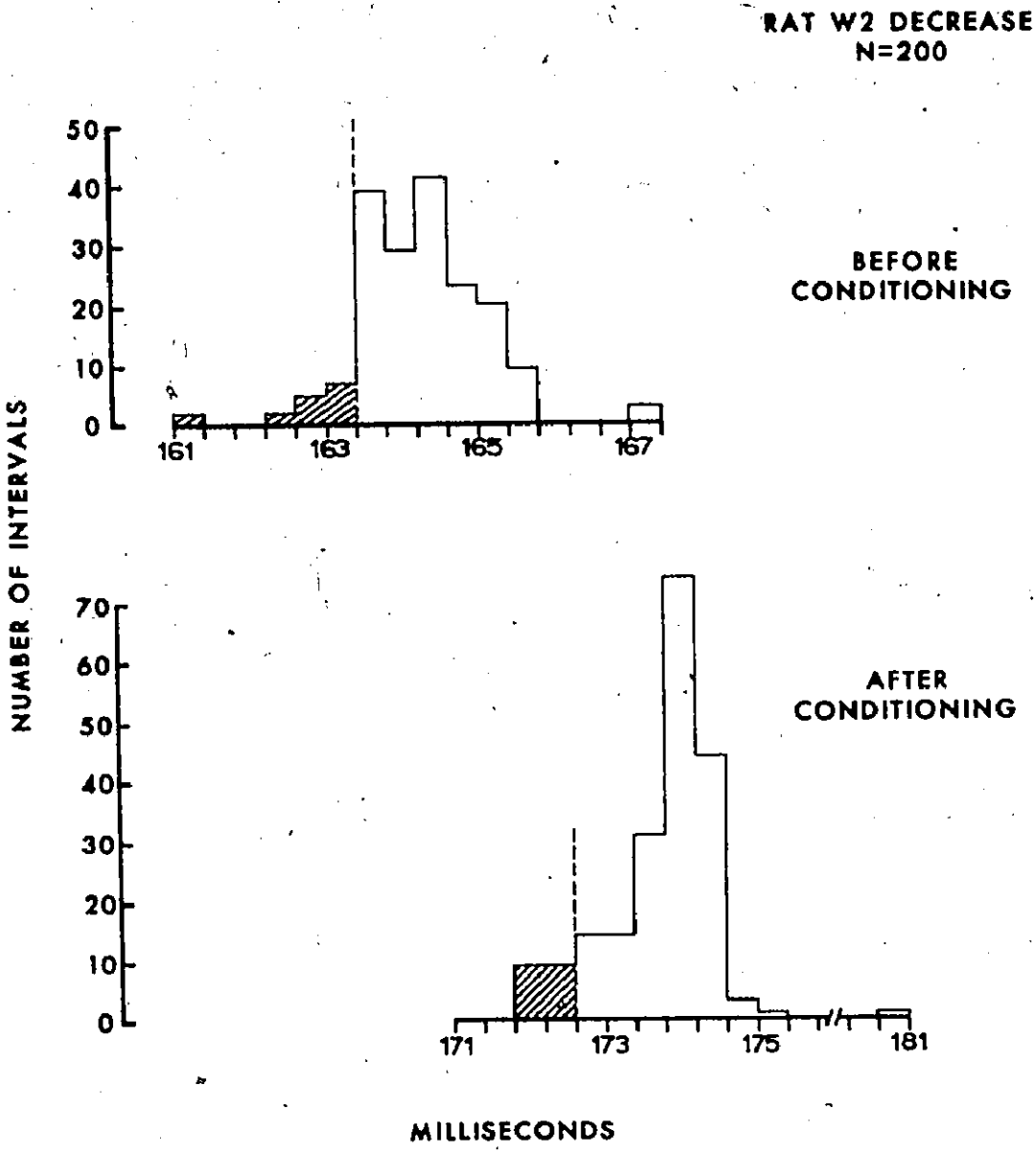
in Figure 2. If the animal was to be trained to decrease its heart rate, the 10th percentile RR interval was chosen as criterion (broken line in Figure 2) and all intervals shorter than this were followed immediately by tail shock. Conversely, if the animal was to be trained to increase its heart rate, the 90th percentile RR interval was chosen as criterion and all responses longer than this value were shocked. Distributions were recompiled and the criterion interval recomputed approximately once per minute, in order to hold the probability of a criterion response and therefore the probability of shock as constant as possible throughout the experiment. If the animal altered its heart rate in the rewarded direction, the criterion was shifted so that once again 10% of the response distribution was being punished. On the other hand, if the animal failed to reach criterion on more than two successive distributions, criterion was relaxed. The experimenter made a consistent effort to shock only 10% of the animal's responses. Animals were run in an alternating order; the first animal was trained to increase its heart rate, the second animal was trained to decrease its heart rate, and so on.

Conditioning was followed by 60 minutes of extinction during which the shock was turned off. Nothing happened to the animal at this time, but all recordings were maintained. At the end of extinction the animal was sacrificed with an overdose of Nembutal.

Results

A total of 24 animals completed conditioning. Ten of these animals were trained to increase their heart rates and 9 were trained to decrease their heart rates. In addition, there were 5 animals that failed to respond electrodermally even when the shock circuits were turned on. It was decided

FIGURE 2 - Distribution of 200 RR intervals before and after punishment for fast heart rates. All intervals falling below the broken line (criterion interval) were punished.


RAT W2 DECREASE
N=200
BEFORE
CONDITIONING
AFTER
CONDITIONING
NUMBER OF INTERVALS
MILLISECONDS
0
10
20
30
40
50
60
70
161
163
165
167
171
173
175
181

at the beginning of the experiment to treat results from these animals separately, to determine whether the presence or absence of electrodermal responding might be a predictor of conditioning success. In all ways these animals received the same conditioning procedure as animals that displayed electrodermal responding. Three of these animals were trained to increase and 2 to decrease their heart rates. An additional 12 rats were discarded prior to the start of training because of heart-rate instability (7), death (3), or equipment problems (2). Six of these 12 rats were also electrodermally inactive, bringing the total number of such rats to 11 or 31% (11/36) of the 36 rats that were paralyzed for heart-rate training.

The findings for all rats that completed punishment training are reported in Table 1, in which heart rate during the first 15 minutes of extinction has been subtracted from heart rate during the last 15 minutes of the operant level period for each rat. When the results were considered, without respect to the status of electrodermal responding, there was no reliable difference between the bidirectional groups on this measure ($t = 1.30$, $p > .05$). However, inspection of the left half of Table 1 reveals that when the electrodermally active animals alone were considered, the bidirectional difference was in the expected direction and statistically reliable ($t = 2.5$, $p < .025$). In contrast, the bidirectional difference for the SP inactive rats was unreliable and in the opposite direction from an operant conditioning effect ($t = -1.35$, $p > .10$).

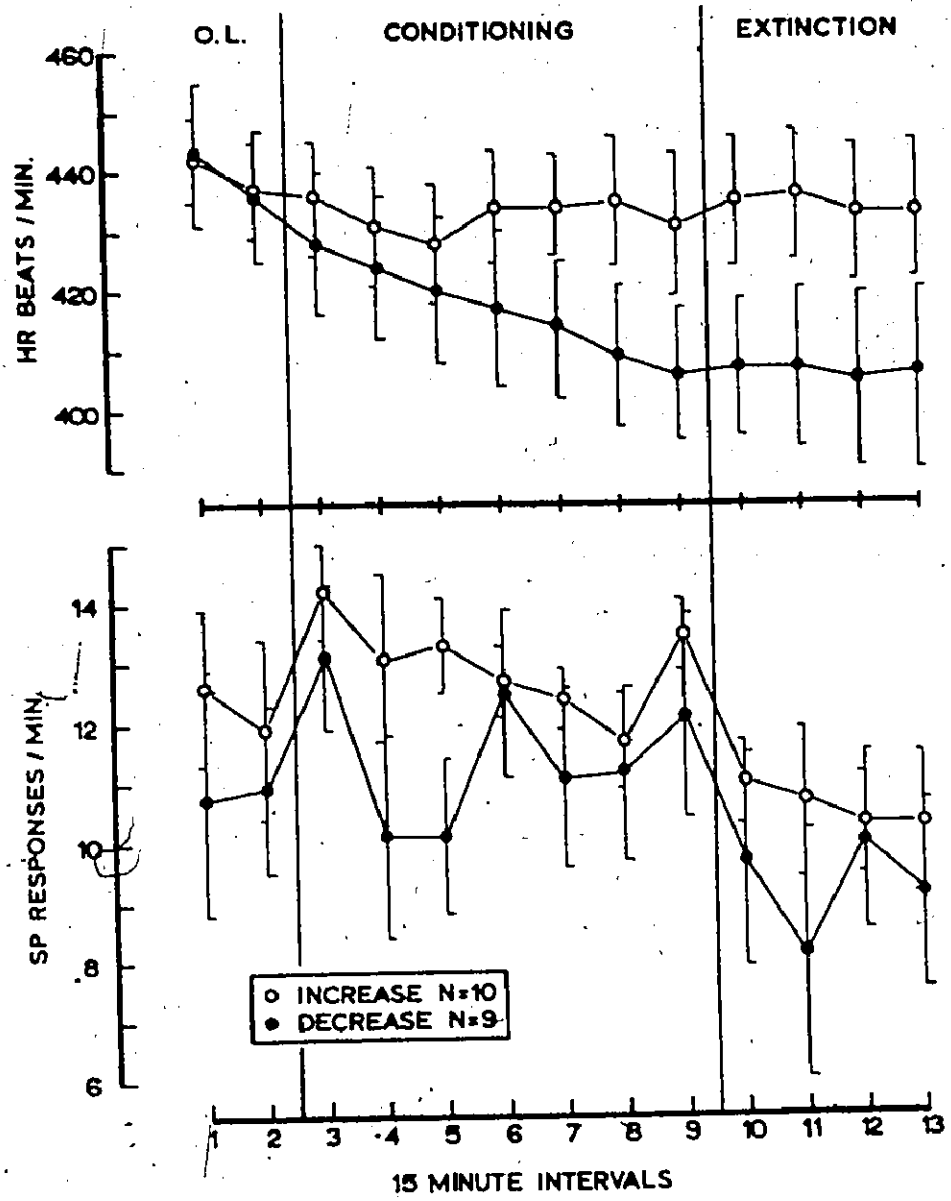
Heart rates of electrodermally active animals were scored from the cardiotachometer channel of the oscillographic record at 15 minute intervals throughout the session and are portrayed in the upper panel of Figure 3. Although both groups were matched for heart rate during the operant level

TABLE 1.- The effect of punishing the RR interval in rats paralyzed by d-tubocurarine. Heart rate during the first 15 min of extinction is subtracted from heart rate during the operant level for each rat.

Table 1

SP Active		SP Inactive	
Increase	Decrease	Increase	Decrease
48	-29	-43	3
23	-36	- 9	-19
-20	-12	-69	
- 9	- 9		
-15	-43		
-33	-46		
25	-60		
- 4	-23		
- 9	1		
-26			
M -2.0	M -28.6	M -40.3	M -8.0
t -0.25	t -2.86	t -1.53	t -0.7
p ns	p < .025	p ns	p ns
t = 2.5		t = -1.35	
p < .025		ns	

FIGURE 3 - Effect of punishing long and short RR intervals on heart rate (upper panel) and electrodermal activity (lower panel) in rats paralyzed by d-tubocurarine. All subjects were electrodermally active.


O.L.
CONDITIONING
EXTINCTION
HR BEATS / MIN.
460
440
420
400
SP RESPONSES / MIN.
14
12
10
8
6
INCREASE N=10
DECREASE N=9
1
2
3
4
5
6
7
8
9
10
11
12
13
15 MINUTE INTERVALS

period, by the end of conditioning rats that were trained to increase their heart rates displayed substantially higher rates than rats that were trained to decrease. However, as a group, rats trained to increase their heart rates actually failed to raise them above their operant level value ($t = -0.25$, $p > .10$). The data reported in Table 1 indicate that only 3 of the 10 rats trained to increase actually did so. Eight out of nine rats trained to decrease their heart rates did so, and this effect was reliable for the group as a whole ($t = -2.86$, $p < .025$). Further inspection of Figure 3 reveals that the bidirectional difference that was apparent at the end of conditioning persisted without diminution throughout 60 minutes of extinction.

The lower panel of Figure 3 depicts the frequency of phasic SP responding during the 60 seconds prior to each heart-rate measure. Response frequency increased reliably for both groups when shock was introduced ($t = 2.39$, $p < .025$ increase group; $t = 2.92$, $p < .01$, decrease group), and decreased reliably for both groups when the shock was terminated ($t = 3.18$, $p < .01$, increase group; $t = 2.18$, $p < .05$, decrease group). Although rats trained to increase their heart rates tended to display higher rates of electrodermal responding than rats that were trained to decrease their heart rates, this difference was not statistically reliable and did not change systematically over the course of conditioning.

Analysis of the peak inspiratory pressure (PIP) of SP active animals indicated that there was no significant difference between the bidirectional groups on this measure. The increase animals received a mean PIP of 22.0 cms of water and the decrease group received 21.2 cms of water ($t < 1$). The PIPs applied to the electrodermally inactive animals ($M = 21.6$ cms of water) did not differ reliably from PIPs received by SP active animals ($t < 1$).

Several analyses were carried out to determine if there was a difference in the number or pattern of shocks received by the bidirectional groups. Two measures were compiled for all rats during each 5 minutes of conditioning. These were a measure of the longest period free from shock (maximum inter-shock interval), and a measure of the longest period of continuous, beat-by-beat shock (maximum punishment duration). In addition, a count was made of the total number of shocks during the final minute of each 5 minute conditioning period. These analyses are presented in Figure 4, where it may be seen that there were no significant differences between the bidirectional groups on any measure. Nor did a reliable difference materialize when statistical tests were applied to the four animals in each bidirectional group that showed the largest heart-rate changes in the expected direction.

Although Figure 4 failed to indicate any difference in the total number of shocks each group received, the relationship of shock density to performance during learning was different for each group. This is apparent in Figure 5 where shock density is plotted as a function of the change in heart rate brought about by conditioning in each bidirectional group. Inspection of this figure indicates that the animals that showed the largest changes in the expected direction were those subjects that received the lower shock densities.

Discussion

In the present study, a bidirectional difference in heart rate was observed only in animals that displayed electrodermal responding. This bidirectional difference developed gradually and persisted throughout 60 minutes of extinction. No systematic differences in skin potential were found between the bidirectional groups, demonstrating that the effect of

FIGURE 4 - Inter-shock interval (upper panel), punishment duration (middle panel) and shock frequency (lower panel) throughout conditioning periods. All rats were paralyzed by d-tubocurarine.

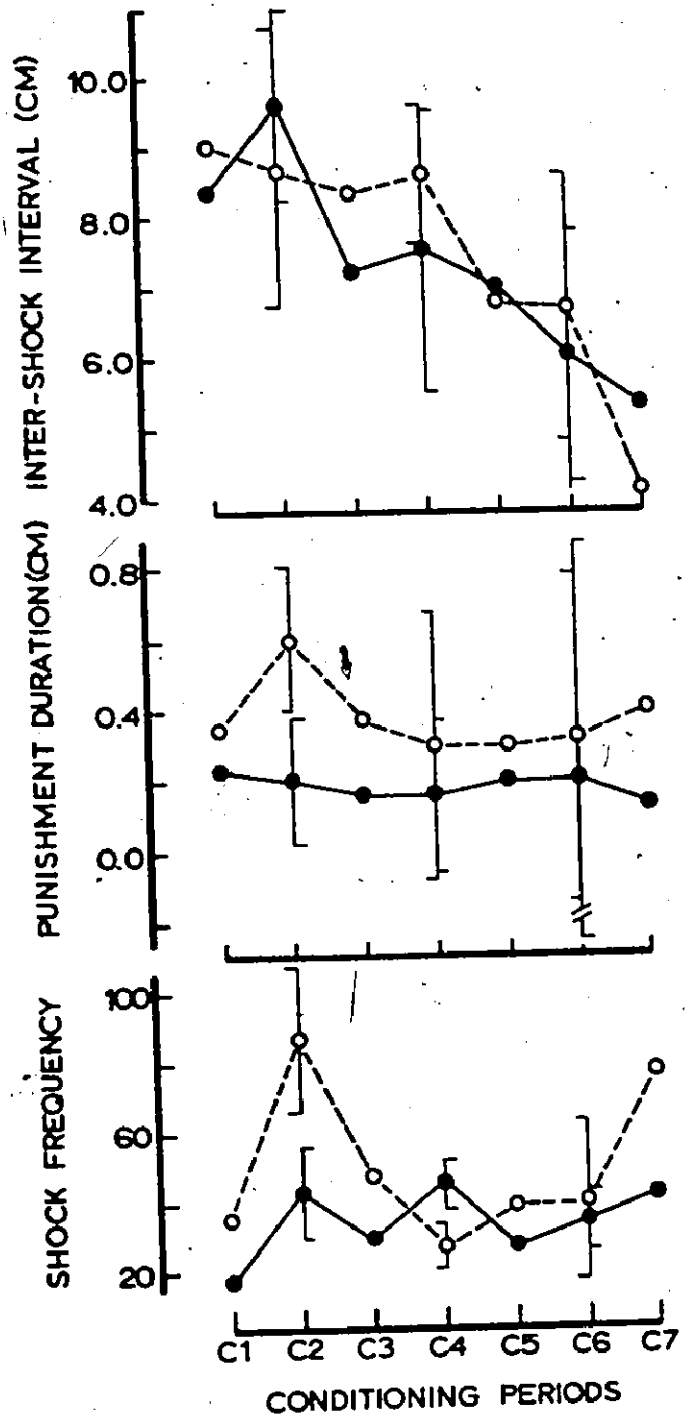
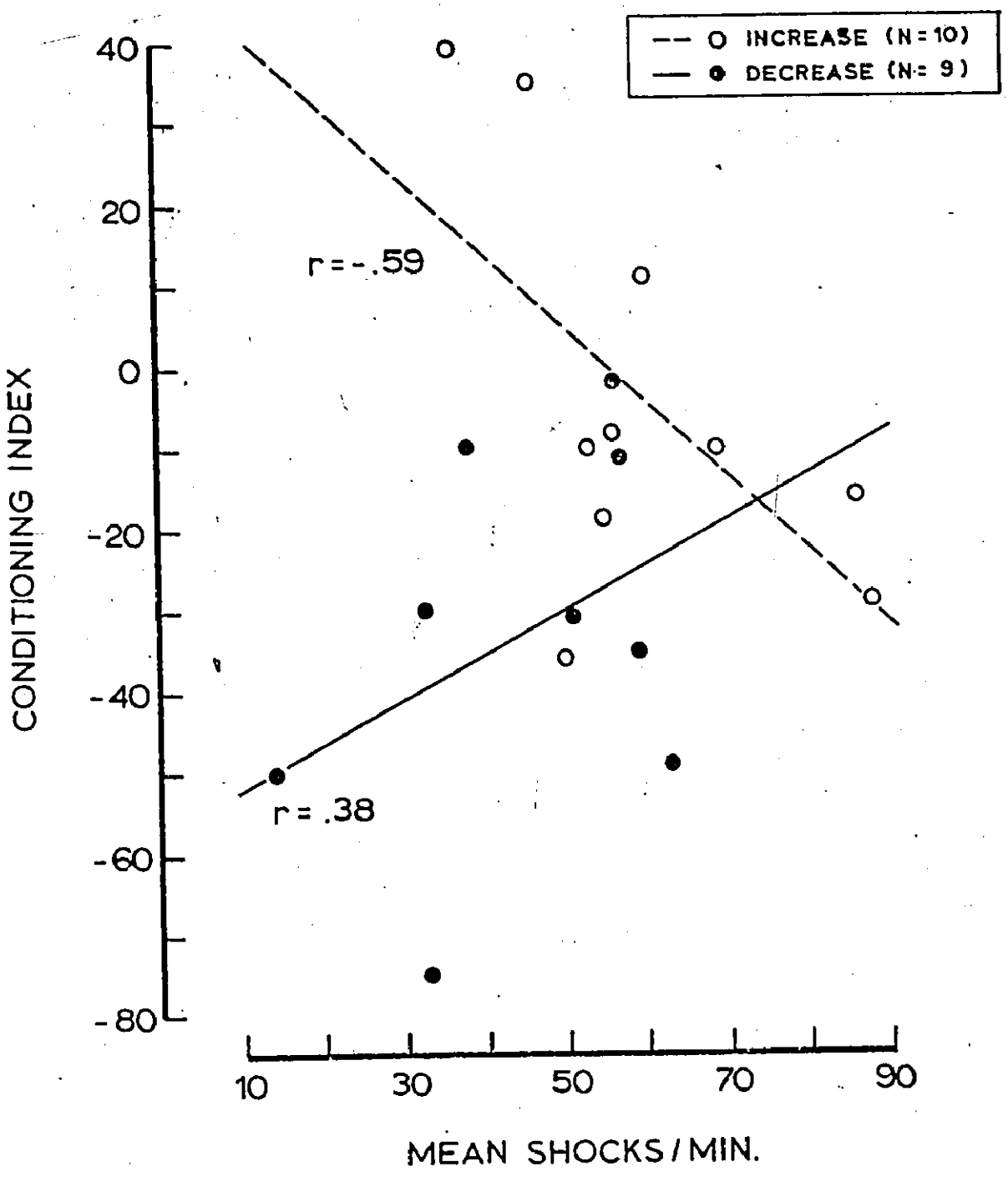

INTER-SHOCK INTERVAL (CM)
10.0
8.0
6.0
4.0
PUNISHMENT DURATION (CM)
0.8
0.4
0.0
SHOCK FREQUENCY
100
60
20
C1
C2
C3
C4
C5
C6
C7
CONDITIONING PERIODS

FIGURE 5 - Changes in heart rate during operant conditioning as a function of shock density for each bidirectional group. Heart rate during the last 15 min of operant level has been subtracted from heart rate during the first 15 min of extinction.


INCREASE (N=10)
DECREASE (N= 9)
r = -.59
r = .38
40
20
0
-20
-40
-60
-80
CONDITIONING INDEX
10
30
50
70
90
MEAN SHOCKS / MIN.

punishment for long or short RR intervals was specific to heart rate.

While these results could be interpreted to indicate that operant conditioning had taken place, the performance of animals in the present study differed in some aspects from the findings reported in earlier punishment studies. Only 3 of the 10 rats trained to increase their heart rates actually did so, whereas 8 of the 9 rats trained to decrease their heart rates displayed lower rates by the end of punishment training. These findings are discrepent from those reported in earlier studies (Fields, 1970b; Hothersall & Brener, 1969), where heart-rate changes in the rewarded direction were obtained in most if not all subjects in both bidirectional groups. A more serious discrepency is encountered when the extinction data are considered. Operantly-conditioned responses are usually expected to diminish in frequency when reinforcement for responding is discontinued. Extinction of learned heart-rate changes was reported in previous experiments that carried out extinction sessions (Hothersall & Brener, 1969; Fields 1970b; Hahn & Slaughter, 1970). However, bidirectional differences did not extinguish in the present study, even though the duration of extinction equalled or exceeded that of previous reports. This raises the question of whether the changes in heart rate that occurred in this experiment were attributable to the same process that generated bidirectional differences in earlier studies of operant heart-rate conditioning in curarized rats.

The relationship of heart-rate changes to shock density observed in the present study may have some bearing on the question of whether these changes were produced by a learning process. The nature of this relationship was that in each bidirectional group rats that displayed the largest changes in heart rate in the direction of training received the lowest shock densities.

These results could be interpreted to indicate that learning had taken place and that shock density was an important determinant of conditioning success. Alternatively, these findings might reflect a tendency on the part of the experimenter to administer more shock to those animals that were not performing well. Unfortunately, it is impossible to determine which of these interpretations is correct on the basis of the current results.

An unexpected finding of the present study was that 31% of the subjects given 3.6 mg/kg of curare became electrodermally unresponsive during adaptation or punishment training. By the end of conditioning, these subjects failed to display reliable heart-rate differences in the expected direction. The mechanism of this effect is not clear. One interpretation is that the bidirectional difference in heart rate shown in Figure 3 was sympathetically mediated and did not occur when peripheral sympathetic ganglia were blocked by an overdose of curare, as may occasionally happen when a large dose of this drug is used (Guyton & Reeder, 1950). Alternatively, electrodermal inactivity may be indicative of a physiological or CNS state, such as shock or unconsciousness, that retarded the progress of conditioning or prevented it altogether.

An additional finding of the present study which was unreported or received little attention in earlier work (Hahn, 1970) was that a large proportion of animals paralyzed with 3.6 mg/kg of curare were unsuitable for experiments on learning. In this study, 12 of the 36 animals that were injected either died before conditioning began or displayed heart rates that were so erratic that punishment training could not be carried out. Many of the remaining animals showed unusually high heart rates, characterized by very little beat to beat variability and a constant decline in baseline rate over the entire session. Further, 1/3 of the subjects were electrodermally

unresponsive. Although the basis of these results is not clear, the factors involved may be responsible for current difficulties many researchers are having in demonstrating operant HR conditioning in paralyzed rats.

CHAPTER THREE

EXPERIMENT 2

The results of Experiment 1 suggested two directions for further work. The first was to attempt another operant conditioning experiment to see whether shock density and the status of electrodermal responding were related to performance during learning. The second approach was to examine and attempt to improve the paralyzed preparation. Since 1/3 of the subjects run under the conditions of Experiment 1 were electrodermally inactive or displayed unstable heart rates that were unsuited for training, the second of these approaches was followed.

The problems encountered when rats are paralyzed by curare fall into two categories. The first concerns the cardiovascular effects of positive pressure ventilation. Positive intrathoracic pressure, encountered when this type of artificial respiration is used, can be expected to compress the venous bed and impair venous return with a subsequent decrease in cardiac output (Brener et al., 1974). Further, respiration parameters are known to be important determinants of the gas composition of arterial blood (Hahn et al., in press). These factors will receive further attention in a subsequent chapter of this thesis, where additional experiments on operant heart-rate conditioning are discussed.

The second problem concerns the influences of the paralyzing agent itself on cardiovascular and CNS functioning. D-tubocurarine is responsible for a variety of effects in addition to its well known actions at the

neuromuscular junction. The research to be discussed in this chapter will address only the second of these two problems.

Some effects of curariform drugs on autonomic and central nervous system (CNS) activity are reviewed in the section that follows. Then, an experiment comparing the effects of two curariform agents on cardiovascular and electrodermal functioning is reported.

Drug Effects

Curariform agents have as their major action the blocking of neural transmission at the skeletal neuromuscular junction. These drugs are classified on the basis of the primary mechanism by which they produce this effect.

D-tubocurarine induces paralysis by combining with the cholinoceptive sites on the postjunctional membrane, thereby blocking the transmitter action of acetylcholine. D-tubocurarine, therefore, is classified as a competitive or anti-depolarizing blocking agent. In contrast to the competitive block, another group of drugs exists whose blocking action produces a persisting depolarization of the motor end-plate. Succinylcholine chloride is a member of this group of depolarizing blocking agents. However, in certain animal species, one of which is the rat, this drug produces a type of block that combines the features of both competitive and depolarizing agents as well as some characteristics not associated with either group (Zaimis, 1953). This type of action is termed a "dual" mechanism.

As d-tubocurarine and succinylcholine differ in their relative mechanism of action, so do they display differences in the sequences and characteristics of paralysis. Animals paralyzed with d-tubocurarine first display motor

weakness and ultimately the muscles become totally flaccid. The small rapid moving muscles (fingers, toes, ears, eyes) are involved first, then the muscles of the limbs, neck and trunk and finally the diaphram becomes paralyzed (Hunt & Kuffler, 1950). With adequate artificial respiration the recovery of muscles will occur in the reverse order to that of their paralysis. Succinylcholine initially causes transient muscular fasciculations over the chest and abdomen, then the neck, arm and leg muscles are involved.

In addition to blocking the neuromuscular junction, both types of curariform agents have additional sites of action and other influences on the organism. One drug action that has received considerable attention in recent years is the effect of these agents upon the autonomic ganglia. Other problems concern the release of histamine and impairment of lung compliance by some neuromuscular blocking agents, and the effect of these agents on CNS functioning.

Ganglionic Blockade

Guyton & Reeder (1950) demonstrated that autonomic ganglia exhibit differential sensitivity to the blocking action of d-tubocurarine. Using anethesized dogs, they found that the vagal innervations to the heart were more readily blocked by this drug than were the sympathetic ganglia. Even though both pathways were affected, the vagolytic action of d-tubocurarine was present at 1.5 times the paralyzing dose, whereas the sympatholytic effect did not appear until 10 times the paralyzing dose had been infused.

Black (1967) also found that the cardiac innervations were affected by d-tubocurarine in dogs. He stimulated the cardiac nerves by means of chronically implanted electrodes so that the vagolytic and sympatholytic effects of d-tubocurarine could be assessed without anesthesia. Stimulating

electrodes were implanted on the vagus nerve in one dog and on sympathetic fibers near the caudal cervical ganglion in a second dog. Depth of curarization was manipulated by controlling rate of infusion and was monitored by recording EMG. Stimulation of the vagus produced a deceleration in heart rate under medium curarization. However, as depth of curarization increased, this deceleration gradually diminished and in fact changed to an accelerative response when total EMG block was achieved. Black interpreted this result to mean that sympathetic fibers contained in the vagus (a mixed nerve) were more immune to the effects of d-tubocurarine than parasympathetic fibers in this dog. The heart-rate response to sympathetic stimulation in the second dog was abolished during the transition to deep curarization. Black concluded that d-tubocurarine blocked peripheral autonomic ganglia in dogs. However, the reversal of the heart-rate response to vagal stimulation suggested that sympathetic fibers were somewhat less affected by d-tubocurarine than parasympathetic fibers, at doses that induce total EMG block.

A recent experiment by Howard et al. (1974) assessed the effect of three neuromuscular blocking agents on the HR response to direct nerve stimulation in the cat. Either d-tubocurarine, succinylcholine, or dimethyl tubocurarine was infused until complete cessation of EMG was obtained. The infusion was then terminated, and the animal was permitted to recover from paralysis. Although they found no significant differences in the heart-rate response to sympathetic nerve stimulation among the drug groups, the baseline heart rate of animals receiving d-tubocurarine was reduced 30 bpm from the pre-drug level by a dose that induced total EMG block. The effect of curariform paralysis on the heart-rate response to vagal stimulation was more profound. However, this effect depended upon which paralyzing drug was used. D-tubocurarine

completely blocked the heart-rate response to vagal stimulation. Although the groups receiving succinylcholine and dimethyl tubocurarine also showed a slight attenuation of the heart-rate response, the effect was small and not reliable in either group. Howard et al. (1974) concluded that d-tubocurarine was the least desirable compound for use in autonomic conditioning experiments because of its profound vagolytic effect.

A somewhat different result has been reported for rats paralyzed with d-tubocurarine. Hahn (1974) exposed the vagus nerve in rats that were anethesized with Nembutal. The subjects were then paralyzed with 3.0 mg/kg d-tubocurarine and the vagus was stimulated directly. Interestingly, he found that a complete vagal block was not present in all animals even though he used a dose of d-tubocurarine that others have reported to totally abolish EMG (Miller & DiCara 1968). He estimated the overall impairment to be about 20% with some animals totally blocked and others not blocked at all. As Hahn did not record EMG, this variability might reflect inadequate curarization. However, Dworkin (1974) recently reported preservation of vagal restraint in rats paralyzed with 8.0 mg/kg d-tubocurarine. This raises the possibility that the effect of this drug is different in the rat than in the cat or dog. The question of whether a species difference exists when succinylcholine or dimethyl tubocurarine is administered cannot be answered at this time as the effect of these drugs on autonomic ganglia has not been assessed in the rat.

Histamine Release

Another important drug action of d-tubocurarine concerns the release of histamine. The histamine releasing properties of this drug are well documented in humans and in the cat (Alam et al., 1939; Comroe & Dripps, 1946). The release of histamine could cause bronchospasm and excessive salivary and tracheobronchial secretions.

Grob (1967) compared the histamine releasing activities of a number of neuromuscular blocking agents. He confirmed that administration of the antidepolarizing blocking agents, such as d-tubocurarine and dimethyl tubocurarine, caused the release of histamine. Interestingly, he concluded that the histamine liberating properties of the depolarizing blocking agents, such as succinylcholine, appeared to be negligible.

Lung Compliance

Massion (1957) reported that curare leads to a decrease in the elastic properties of the thorax. Using dogs paralyzed with d-tubocurarine, he measured a decrease of 42.2% in lung compliance and a 5.9% decrease in the compliance of the rib cage. He concluded that if parameters of artificial respiration were not adjusted to correct for changes in elasticity, then hypoventilation may result.

More recently, Brener et al. (1974) measured circumferential chest movements and heart rate in rats paralyzed with d-tubocurarine. He found that respiration settings that were sufficient to maintain the animal in a healthy condition at the beginning of the session, lead to diminishing chest circumference and fatal hypoventilation in 75% of the subjects by the 180th minute of curarization. It is possible that this decline in chest circumference reflected a decrease in tidal volume caused by the loss of lung compliance. Brener found that when respiration settings were altered throughout the session to maintain a constant chest circumference on each respiratory cycle, heart rate also remained stable.

CNS Effects

The effect of curariform agents upon the central nervous system is not clear. For many years, experimenters assumed that curariform drugs did not

affect the CNS and that the subjects were conscious. This assumption was based upon a handful of studies using human subjects. In one of these studies, Smith, an anesthestist, permitted himself to receive two and one-half times the amount of d-tubocurarine necessary for paralysis of all skeletal muscles. Included among the functions examined were EEG, EKG, memory, vision, hearing, and pain threshold. Smith et al. (1947) concluded that, even in large doses, d-tubocurarine had no significant central action in man. In another study (Leuba et al., 1968) human subjects who had undergone total paralysis by d-tubocurarine complained of mucous build up during the period of paralysis and felt excessively tired after motor function had recovered. However, these subjects performed mathematical tasks during paralysis and reported afterwards that they experienced no impairment of memory or clouding of consciousness. The result of this and other human experiments (Unna et al., 1950) suggest that neuromuscular blocking agents when administered in doses that induce flaccid paralysis are devoid of central effects.

Results from the animal experiments are more confusing. Studies monitoring the EEG of paralyzed animals have reported everything from arousal to slow-wave sleep patterns. Black, Carlson and Solomon (1962) concluded that the EEG pattern of curarized animals was affected by species, dose level, method and speed of injection, as well as by the adequacy of artificial respiration. In another study, Hodes (1962) assessed the CNS effects of three neuromuscular blocking agents. He found that all of the drugs tested produced an EEG pattern indicative of sleep, after a short period of time. Early in the session, mild stimuli produced an arousal reaction, but as time under paralysis increased, even noxious stimuli failed to desynchronize the EEG. In contrast to these results, Roberts (unpublished observations) was unable to demonstrate

a clear effect of d-tubocurarine (1.2 mg/kg) or succinylcholine (4.0 mg/kg) on evoked potentials recorded from the hippocampus, amygdala, preoptic nucleus, ventromedial nucleus or anterior neocortex in the rat. However, those observations were based on a total of only seven rats.

In summary, no clear statement about the CNS effects of d-tubocurarine seems possible at this time. It is pertinent to note that quaternary compounds, such as d-tubocurarine, are not expected to penetrate the blood brain barrier (Koelle 1965). Thus some of the discrepencies in the earlier reports may reflect the effect of prolongued artificial respiration on CNS functioning, rather than the pharmacological effects of the paralyzing drug.

Summary

Several studies have examined the effects of curariform agents on cardiovascular and CNS functioning. These studies have shown that d-tubocurarine blocks autonomic ganglia in the dog and the cat. The parasympathetic fibers are especially sensitive to blockade, which appears to be complete at doses of d-tubocurarine that abolish EMG. The effect of d-tubocurarine on vagal function in the rat, however, is less clear. Although some impairment of vagal outflow has been demonstrated in this species, there is reason to believe that considerable vagal restraint is maintained at doses that have commonly been used in studies of operant heart-rate conditioning.

The available research also indicates that administration of d-tubocurarine leads to release of histamine and a progressive decrease in lung compliance. These factors are likely to produce a conjested rat and a decreased tidal volume with hypoventilation as the result. The evidence for the central effects of d-tubocurarine in the rat is contradictory and at the present time no clear statement about the effect of curariform agents on the CNS is possible.

The data currently available suggest that other curariform drugs, particularly succinylcholine, have less potent vagolytic and histamine releasing effects and are probably better suited for studies of learning in the rat. However, the effect of different paralyzing drugs on heart rate has not been evaluated in this species. Some data pertinent to this issue are presented in the next experiment.

Experiment 2

The objectives of the present experiment were threefold. The first was to compare the effects of d-tubocurarine and succinylcholine on heart rate in the rat. If the high invariant heart rates found in Experiment 1 were the result of the vagolytic effects of d-tubocurarine, then paralysis by succinylcholine, a drug with minimal vagal effects, might be expected to reduce heart rate and increase heart-rate variability. The second goal was to determine whether there was retention of vagal restraint at doses of d-tubocurarine and succinylcholine that induced flaccid paralysis. This was accomplished by administering atropine, a parasympathetic blocking agent, to rats that were paralyzed by d-tubocurarine or succinylcholine.

The third goal of the present study was to examine the effects of both paralyzing agents on sympathetic functioning. Possible effects on this system were evaluated by recording skin potential, a response that is determined entirely by sympathetic outflow. The first experiment suggested that sympathetic functioning was impaired by 3.6 mg/kg d-tubocurarine, as this dose rendered 1/3 of the animals electrodermally unresponsive.

Method

Subjects

The subjects were 50 male hooded rats from Quebec Breeding Farms stock, ranging in weight from 360 to 470 gms. The animals were divided into five groups. Two groups were paralyzed by either a high or low dose of d-tubocurarine, whereas two additional groups received a high or a low dose of succinylcholine. The fifth group was a saline control that was not paralyzed at all.

Electrophysiological Recording

All animals were implanted with EKG electrodes subdermally on either side of the rib cage. Wire leads ran from these electrodes beneath the skin to an Amphenol connector which was cemented to the animal's skull. Implanting HR electrodes subdermally allowed heart rate to be recorded in the normal state. All animals were permitted 7 to 10 days to recover from surgery and were handled for the final 3 to 5 days of this period.

Electrodermal electrodes were preselected and prepared as in Experiment 1. The active electrodes were placed so as to encapsulate the four interdigital pads on the rear feet and were referenced to two abraded sites on the tail.

Drugs

Two preliminary experiments were conducted on separate groups of animals to evaluate effective doses of atropine sulphate (Parke & Parke, 1 mg/cc) and succinylcholine chloride (Anectine, Burroughs-Wellcome Co., 20 mg/cc).

The first experiment explored a variety of doses of atropine ranging from 1.0 mg/kg to 4.0 mg/kg. Heart rate and skin potential were recorded throughout a 70 minute testing session. All animals received an injection of 0.2 cc isotonic saline 20 minutes after recording began. Following an additional 20 minutes each rat received one of four doses of atropine

(1.0 mg/kg, 1.5 mg/kg, 2.0 mg/kg or 4.0 mg/kg). Each of the six animals in the experiment was tested on two doses of atropine with a four day interval between testings. Half the animals were tested on a low dose first, and then on a higher dose. This order was reversed for the remaining subjects.

The effect of atropine on heart rate and skin potential is presented in Figure 6. Inspection of this figure reveals that administration of atropine caused a substantial increase in heart rate and abolished skin potential responding at all doses tested. The effect on both heart rate and skin potential was slightly greater at the higher doses. Although there was little basis for preferring one dose to another, 2.0 mg/kg was chosen for use in subsequent experiments to induce a substantial vagal blockade.

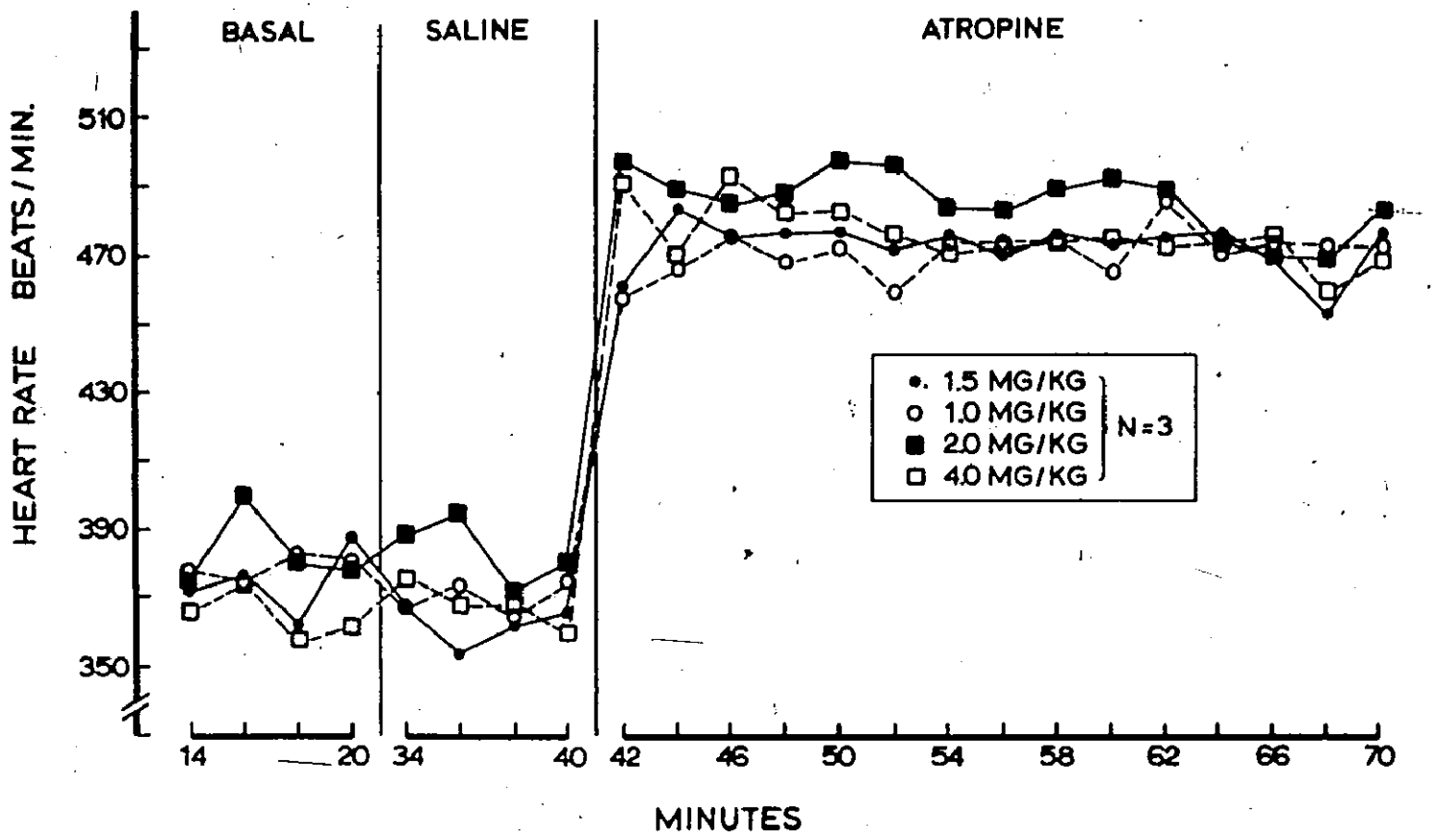
A second preliminary experiment was carried out on an additional sample of 18 animals to determine a high and a low dose of succinylcholine. The selection of the lower dose was based upon an initial injection and subsequent infusion that rendered the animal flaccid and removed any overt movement to tactile stimulation for the duration of testing (2 hours). Animals receiving the lower dose of 4.0 mg/kg succinylcholine were infused at 1.6 mg/hr, which corresponded to a nominal infusion of 4.0 mg/kg/hr for a 400 gm rat. Animals receiving the higher dose of 12.0 mg/kg were infused at 3.2 mg/hr (nominal infusion of 8.0 mg/kg/hr).

Animals injected with d-tubocurarine received 1.2 or 3.6 mg/kg as an initial dose, and were infused at 0.4 mg/hr (1.0 mg/kg/hr) or 0.8 mg/hr (2.0 mg/kg/hr). All initial injections were i.p. and all infusions were applied to the right thigh muscle.

Procedure

All animals were partially restrained in a rat holding device similar

FIGURE 6 – The effect of the administration of various doses of atropine upon heart rate.


BASAL
SALINE
ATROPINE
HEART RATE BEATS / MIN.
510
470
430
390
350
14
20
34
40
42
46
50
54
58
62
66
70
MINUTES
1.5 MG/KG
1.0 MG/KG
2.0 MG/KG
4.0 MG/KG
N=3

to the one employed by Roberts (Roberts & Young, 1971). The restraining stand was modified to enable the respirator mouthpiece to be brought into view and secured in front of the animal. The respiration technique employed the same parameters as in Experiment 1.

Each subject was placed in the restraining stand for an adaptation session prior to the experiment. Nothing happened to the animal during this time, and it was returned to its home cage after 30 minutes. Two days later, the animal was placed in the restraining stand again for testing.

Twenty minutes after recording began, each rat received 0.2 cc i.p. injection of isotonic saline. Twenty minutes after this they received either d-tubocurarine (1.2 or 3.6 mg/kg), succinylcholine (4.0 or 12.0 mg/kg) or another injection of saline. Rats receiving curare or succinylcholine were respirated and infused as described previously. Thirty-five minutes after paralysis all animals received an injection of 2.0 mg/kg atropine. Recordings were maintained for an additional 30 minutes after which the animal was sacrificed with an overdose of Nembutal.

Results

Three animals receiving 3.6 mg/kg curare either died or displayed such erratic heart rates that they could not be maintained. No animal receiving 1.2 mg/kg curare or either dose of succinylcholine was discarded. However, an additional subject was discarded as its atropine injection failed to affect electrodermal or heart-rate responding. A subsequent injection was effective suggesting that the first was badly placed.

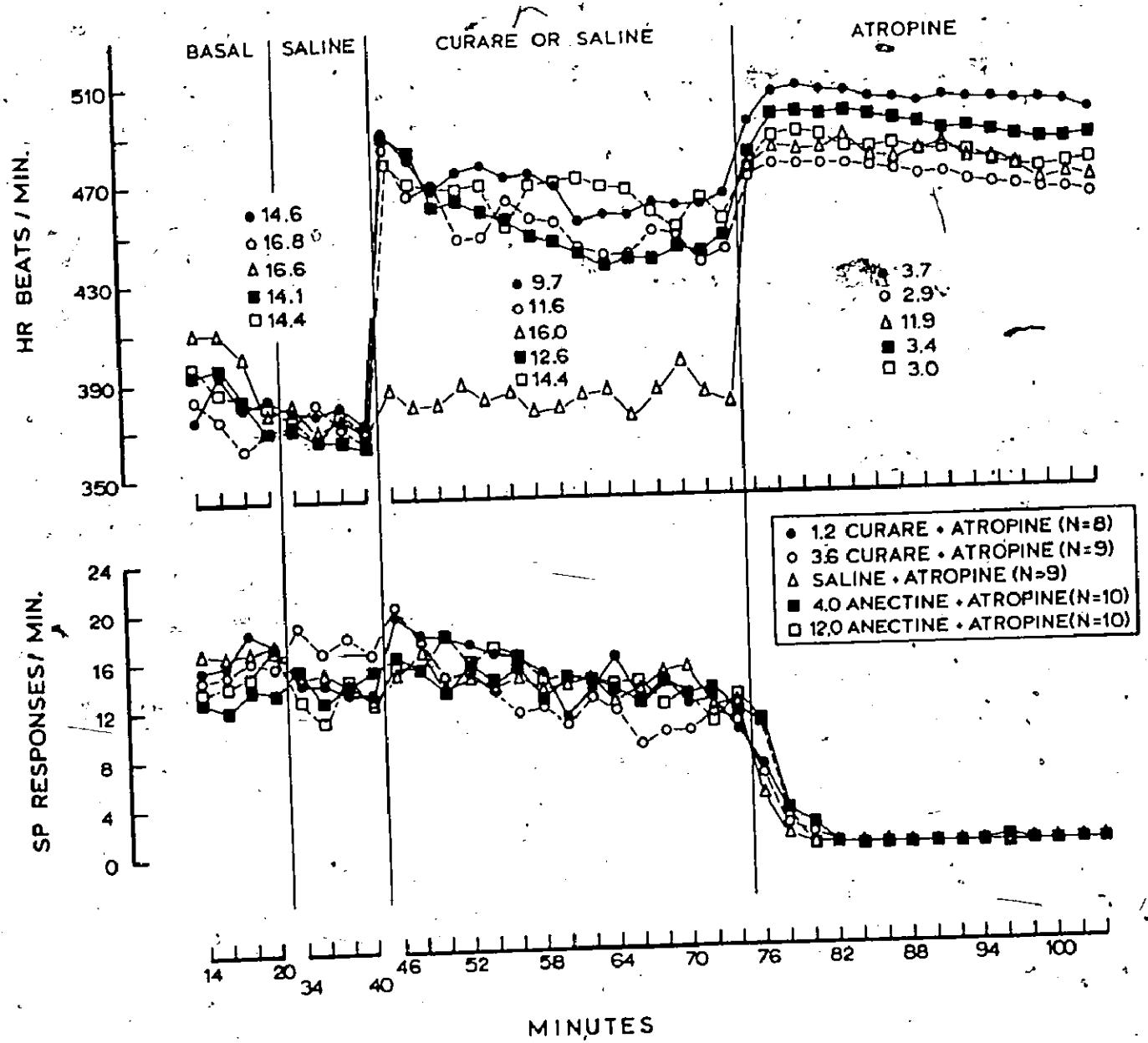
The Effect of Paralysis on Heart Rate

The heart-rate results are presented in the upper panel of Figure 7.

The measure is the mean heart rate for all subjects, scored at 2 minute intervals from the cardiotachometer channel of the polygraph record. Administration of either d-tubocurarine or succinylcholine increased heart rate from 370 to 480 bpm ($p < .01$) after which a gradual decline occurred. Atropine subsequently raised heart rate to a level (again 480 bpm) that was not substantially different from the heart rates displayed by rats given atropine without a paralyzing drug. With one exception, there were no differences in heart rate among the four doses of paralyzing drugs at any point in the experiment. The exceptation was that, following atropinization, rats previously given 1.2 mg/kg curare were found to have significantly higher heart rates than rats given 3.6 mg/kg curare ($t = 3.14$, $p < .01$).

Within-subject standard deviations in heart rate are also reported in Figure 7. These were based on eight HR measures taken at 2 minute intervals for each rat. Measurement began shortly after the start of testing when pre-drug variability was assessed, and approximately 30 minutes after injection when variability following a paralyzing drug or atropine was measured. Paralysis by curare was followed by a moderate reduction in heart-rate variability ($t = 2.7$, $p < .01$) whereas succinylcholine had a smaller and statistically unreliable effect on this measure ($t < 1$). Atropine, however, sharply reduced HR variability in rats given curare ($t = 4.46$, $p < .001$) or succinylcholine ($t = 6.32$, $p < .001$). The combined action of atropine and either paralyzing drug on heart rate variability was more potent than atropine alone. Similar results were found when standard deviations were calculated for 11 measurements taken at 4-second intervals at the conclusion of each phase of testing, although these data are not reported in Figure 7.

FIGURE 7 - Effect of curare plus atropine and succinylcholine plus atropine on heart rate (upper panel) and electrodermal activity (lower panel). The numbers in the upper panel are mean within-subject standard deviations (basal and saline periods are combined).


BASAL
SALINE
CURARE OR SALINE
ATROPINE
HR BEATS / MIN.
510
470
430
390
350
● 14.6
○ 16.8
△ 16.6
■ 14.1
□ 14.4
● 9.7
○ 11.6
△ 16.0
■ 12.6
□ 14.4
● 3.7
○ 2.9
△ 11.9
■ 3.4
□ 3.0
SP RESPONSES / MIN.
24
20
16
12
8
4
0
● 1.2 CURARE + ATROPINE (N=8)
○ 3.6 CURARE + ATROPINE (N=9)
△ SALINE + ATROPINE (N=9)
■ 4.0 ANECTINE + ATROPINE(N=10)
□ 12.0 ANECTINE + ATROPINE(N=10)
14
20
34
40
46
52
58
64
70
76
82
88
94
100
MINUTES

The Effect of Paralysis on Skin Potential

The lower panel of Figure 7 indicates that the effect of paralysis on electrodermal activity was quite different from its effect on heart rate. Although a substantial proportion of animals paralyzed with 3.6 mg/kg curare failed to display electrodermal responding in Experiment 1, spontaneous activity was present in all subjects given this dose in the present study. Animals receiving the high dose of curare did not differ significantly at any point in the experiment from rats given 1.2 mg/kg curare or either dose of succinylcholine, or from non-paralyzed controls. However, paralysis by 3.6 mg/kg curare was followed by a statistically reliable decrease in electrodermal responding below the precurare rate ($t = 2.07$, $p < .025$), whereas paralysis by other drugs was not. Furthermore, 3 animals given 3.6 mg/kg curare exhibited a reduction in skin potential frequency of more than 50%, compared to baseline responding. One animal displayed a reduction of 83%. These results suggest that some rats receiving 3.6 mg/kg curare might have become electrodermally unresponsive had they been studied for a longer period of time, as was done in Experiment 1.

The same pattern of results was obtained when the amplitude of SP responding was analyzed. Paralysis by 3.6 mg/kg curare resulted in the largest decrement in SP amplitude but this effect was not reliable ($t < 1$). None of the other drugs had any effect on this measure.

Atropine abolished electrodermal responding in all groups. This was expected as the postganglionic sudomotor fiber is cholinergic in the rat (Roberts & Young, 1971).

Discussion

In the present study, all animals that were paralyzed with either

curariform agent displayed elevated heart rates that were markedly deviant from the heart rates of non-paralyzed controls. However, the effect of atropine upon heart rate indicates that the elevated baselines were not the product of total vagal blockade. Administration of atropine caused a large increase in heart-rate baseline and a reliable decrease in heart-rate variability in all groups. This indicates that substantial vagal function is retained or has recovered after 30 minutes of curarization, even when 3.6 mg/kg d-tubocurarine is administered.

The fact that succinylcholine and d-tubocurarine affected baseline heart rates in a similar way suggests further that vagal blockade was not primarily responsible for the increase in heart-rate baseline. Although the effect of succinylcholine on the vagus has not been evaluated in the rat, it is devoid of vagolytic actions in other species.

While both curariform agents affected heart-rate baselines similarily, succinylcholine and d-tubocurarine did have differential effects on heart-rate variability. Paralysis by both doses of d-tubocurarine reduced variability, whereas paralysis by succinylcholine had a negligible effect on this measure. One interpretation of this result is that heart-rate variability is more sensitive to vagal blockade than is the heart-rate baseline, and that the differential effect of d-tubocurarine and succinylcholine reflects differences in the vagal blocking properties of these drugs. This could account for the finding that heart-rate variability was reduced more in subjects that received atropine plus d-tubocurarine, than in those animals that received atropine alone. However, if this interpretation were correct, then a greater decrement in heart-rate variability might have been expected in animals that received d-tubocurarine and atropine than in subjects that

received succinylcholine and atropine, but this was not the case. In general, the findings of the present experiment concerning heart-rate variability do not fit well with any unitary explanation which alludes to vagolytic or sympatholytic effects of curariform drugs, or to respiratory function as the source of heart-rate variability.

Electrodermal activity was present in all drug groups. However, response frequency decreased over the session for rats given 3.6 mg/kg d-tubocurarine. Furthermore, three subjects in this group showed a reduction of over 50% in skin-potential response frequency over the course of the experiment. This suggests these animals might have become electrodermally inactive, if the duration of the testing session had been extended. Although the results of this and the previous experiment suggest some proportion of animals paralyzed by 3.6 mg/kg d-tubocurarine can be expected to become electrodermally inactive, the basis of this effect is not clear. This finding may reflect CNS effects of large doses of d-tubocurarine, or it may reflect peripheral effects such as vasoconstriction and subsequent changes in skin temperature which are known to be important determinants of SP responding in several species, including the rat (Roberts, unpublished observations; Edelberg, 1972).

The failure in the present study to observe substantial differences in the heart rates of rats paralyzed by d-tubocurarine and succinylcholine suggests that the cardiovascular state of the animal is not determined primarily by the pharmacological action of the drugs. This implicates positive pressure ventilation as a major determinant of the deviant heart rates displayed by paralyzed rats. Artificial respiration has profound effects on the cardiovascular system and upon the gas composition of arterial

blood (Brener et al. 1974). Future attempts to improve the paralyzed preparation will likely be more successful if attention is concentrated on the variables associated with artifical respiration.

CHAPTER FOUR

EXPERIMENT 3

The role of respiration in determining the cardiovascular state of the curarized rat has received considerable attention recently in light of the current failures to replicate previous findings of operant heart-rate conditioning in the paralyzed preparation (Miller and Dworkin 1974). Experiment 2 suggested that positive pressure ventilation plays a more important part in determining the heart rate of curarized rats than does the pharmocological action of paralyzing drugs.

This chapter begins by reviewing the problems of artificial respiration and then presents a second attempt to operantly condition heart rate in the paralyzed rat.

Artificial Respiration

The positive pressure respiratory system used in the current experiments and in all of the previous work on operant conditioning, employs three parameters to achieve respiratory adequacy. These are respiration rate, peak inspiratory pressure (PIP), and the ratio of time spent in inspiration and expiration during each respiratory cycle (I/E ratio). Until recently, it was believed that respiration parameters of 70 breathes per minute, I/E ratio of 1:1 and a PIP of 20 cms of water (herein abbreviated 70/1:1/20) were adequate to maintain a rat in a normal physiological state (DiCara 1970).

DiCara (1970) determined the adequacy of various respiratory parameters

by analyzing arterial blood gases in normal and paralyzed rats. First, he established normal pCO_2 and pO_2 for the noncurarized rat as 25.4 mm Hg and 75.1 mm Hg respectively. Then the subjects were paralyzed with d-tubocurarine and respirated at 70 breathes per minute, I/E ratio of 1:1 and PIPs of 10, 15 or 20 cms of water. Animals remained at each respiratory setting for 60 minutes. Blood-gas analyses performed on rats ventilated at these parameters revealed that respiration settings of 70/1:1/20, the values used in previous studies of learning, produced arterial blood gases that approximated the pre-curare level.

Blood-gas composition in normal and paralyzed rats has been evaluated more recently by Hahn et al. (in press). The pO_2 and pCO_2 found by Hahn for curarized rats respirated at 70/1:1/20 corresponded reasonably well with those reported by DiCara (pO_2 73 versus 77 mm Hg, pCO_2 21 versus 23 mm Hg respectively). However, blood gas determinations reported for normal state rats were sharply discrepant. Particularly important physiologically was the pCO_2, which is an important determinant of, and is inversely related to, the oxygen content of the blood (Riley, 1966). Hahn and his coworkers found the pCO_2 to be 31.1 mm Hg in the normal state, a value that was considerably higher than the DiCara assessment of 25.4 mm Hg for normal state rats and the pCO_2 of approximately 22 mm Hg reported by both investigators for rats curarized and respirated at 70/1:1/20. This suggests that respiration of a curarized rat at 70/1:1/20 produces disturbances in the chemical composition of arterial blood characterized by a reliable decrement in pCO_2 below the normal state value. Hahn et al. also found that these respiratory settings produced a reliable increase in blood pH and a significant decrease in bicarbonate level over the 70 minute session. Both of these changes are

consistent with a lowered pCO_2 and suggest that respiration at 70/1:1/20 results in a hyperventilated subject in an abnormal cardiovascular condition. The fact that Hahn et al.'s rats displayed heart rates in excess of 500 bpm at the end of testing is consistent with this conclusion.

Hahn also assessed the effect of an alternative set of respiratory parameters on blood-gas composition. These settings consisted of a respiration rate of 60 breaths per minute, an I/E ratio of 1:2 and a PIP of 16 cms of water (60/1:2/16), as recommended by Brener et al. (1974). Respiration of a curarized rat at these settings produced pH, bicarbonate and pCO_2 within the normal range, with a trend toward an increase in pCO_2 over the testing session. However, these respiratory parameters also produced a reliable decrement in pO_2 from approximately 70 to 58 mm Hg within 30 minutes of paralysis. Rats respirated at 60/1:2/16 displayed lower heart rates than animals ventilated at 70/1:1/20, but still had heart rates in excess of 460 bpm after 70 minutes of curarization. As most experiments on autonomic conditioning last longer than 70 minutes, the decrement in pO_2 and the increasing trend of pCO_2, together with a probable decrease in lung compliance discussed earlier, suggest that rats respirated at 60/1:2/16 would eventually become hypoventilated if counter-measures were not taken.

Gaebelein and Howard (submitted) attempted to produce normal blood-gas values in the paralyzed rat by maintaining peak expired CO_2 at a constant value throughout paralysis. This was accomplished by altering the rate of constant volume respiration and the I/E ratio in such a way as to produce and maintain expired CO_2 at 5%. Rats respirated in this way displayed a relatively normal pCO_2 for up to 90 minutes of curarization. However, Gaebelein and Howard also reported a decrement in pO_2 below the precurare

level that was present after 30 minutes and reliable after 60 minutes of paralysis. Although this procedure increases the pCO_2 to an acceptable value, the low pO_2 suggests that hypoventilation may result with this technique as well.

Brener et al. (1974) attempted to counteract the progressive hypoventilation that occurs when rats are respirated at lower PIPs. They measured heart rate and circumferential chest movements under various respiratory parameters. Rats were paralyzed with d-tubocurarine and respirated at either 70/1:1/18 or 70/1:1/14. Subjects in both these groups displayed high initial heart rates that declined systematically, as did circumferential chest movements, over the 90 minute session. By the end of the experiment, 75% of the animals respirated at 70/1:1/14 had suffered fatal hypoventilation. Brener et al. attributed the decline in chest circumference in both these groups to the loss of lung compliance induced by the injection of d-tubocurarine. A final group, respirated at 60 breaths per minute, and an I/E ratio of 1:1, had PIP adjusted throughout the session to maintain constant chest movements and to counteract the progressive hypoventilation that occurred when respiratory pressure remained fixed. Animals respirated in this manner displayed a stable heart rate, around 440 bpm, over the entire session.

In summary, the current data agree that respiration settings of 70 breaths per minute, an I/E ratio of 1:1 and PIP of 20 cms of water (70/1:1/20), result in a hyperventilated rat with a subnormal pCO_2. However, efforts to normalize the pCO_2 by reducing the PIP and altering the I/E ratio, or maintaining expired CO_2 at a constant value, run the risk of reducing pO_2 values and producing a hypoventilated subject. The technique described by Brener et al. (1974) of readjusting the PIP throughout the session to maintain

constant chest excursions appears to counteract the progressive decrease in lung compliance during prolonged paralysis and produces a stable heart-rate baseline. However, the effect of this procedure on blood gases has not yet been evaluated.

Experiment 3

The third experiment to be reported in this thesis is another attempt to operantly condition heart-rate in the paralyzed rat. This study incorporates several changes suggested by the results of the previous experiments.

One of these changes concerns the paralyzing drug. Succinylcholine was used to immobilize the animals in this experiment for the following reason. Although the differences between succinylcholine and d-tubocurarine were minor in Experiment 2, skin potential responding and heart-rate variability were better preserved under succinylcholine than under d-tubocurarine.

A second change was a modification of the respiration technique. It was decided to adopt Brener's procedure of altering PIP throughout the session to maintain a constant chest circumference on each respiratory cycle. This technique has provided stable heart-rate baselines in previous work (Brener et al., 1974).

In the first operant conditioning experiment, 1/3 of the animals that were paralyzed could not be used as they were either electrodermally inactive, displayed unstable heart rates, or died before conditioning was completed. Based on this finding a decision was made to condition only those animals that met certain requirements before the experiment began. These preselection criteria were a stable heart-rate baseline between 400-480 bpm, a PIP between

12-18 cms of water, a core temperature greater than 35°C, and the presence of electrodermal activity.

In order to increase the likelihood that at least one subject would meet the preselection criteria during each experimental session, the rats were prepared in pairs. The rat not chosen to be an experimental was run as a yoked control. Normally, yoked control data are useful in determining whether operant conditioning occurs in both bidirectional groups or only in one. However, in the present study, the subject chosen to be the yoked control was often the subject that failed to meet the preselection criteria. For this reason, data from the yoked control groups must be interpreted with caution.

An additional change was made to the conditioning procedure itself. During conditioning, each criterion response, and therefore each shock, was followed by an unsignalled time-out period of 200 msecs, during which no shocks were administered. This change was made to reduce shock density, as the results of Experiment 1 indicated that this variable might be an important determinant of conditioning success.

Method

Subjects

The subjects were 38 male hooded rats obtained from Quebec Breeding Farm stock and ranging in weight from 330 to 470 gms. All animals were food and water deprived for approximately 18 hours prior to the experiment.

Three animals died shortly after the onset of paralysis because of a malfunction in one of the respirator valves. An additional four animals were terminated before conditioning as neither the yoked control nor the experimental subject met the preselection criteria.

Procedure

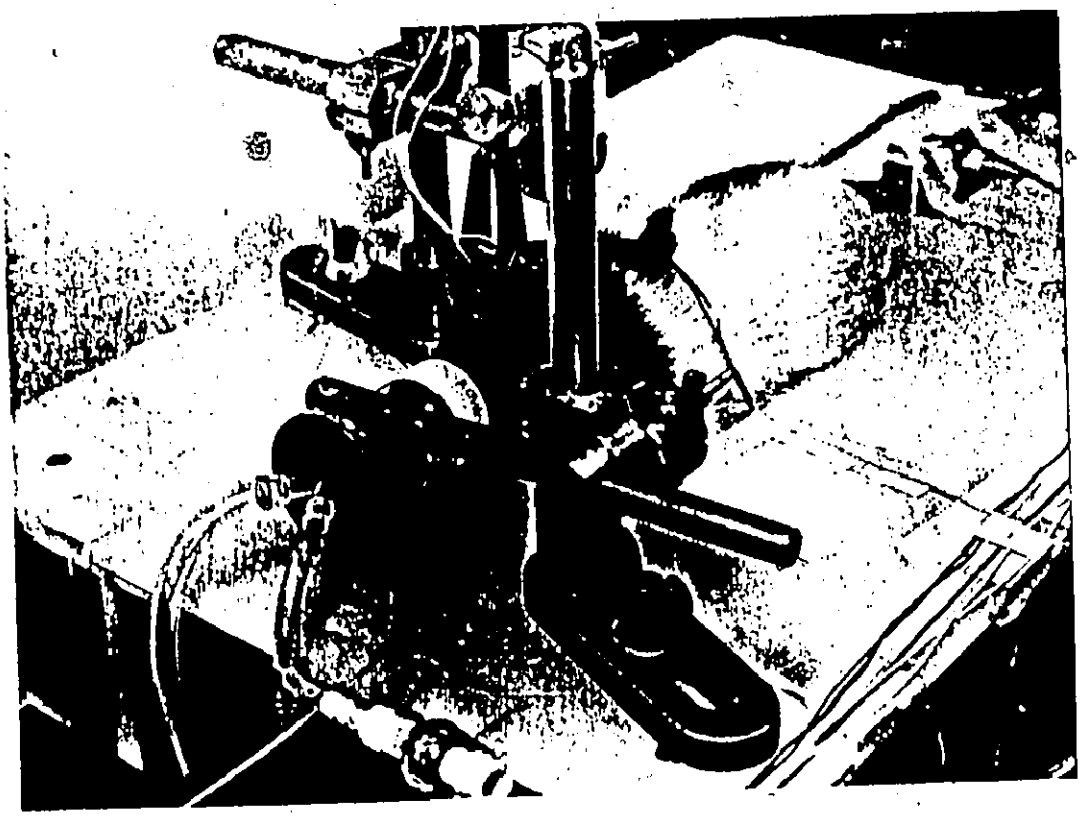
All animals were injected with 4.0 mg/kg succinylcholine chloride (Anectine, Burroughs Wellcome, 20 mg/cc). The injection was placed 1/2 cm below the rib cage as demonstrated by Brener (1972 personal communication). This method induced paralysis within 10 seconds, thus permitting immediate artificial respiration and reducing possible trauma to the cardiovascular system.

The physical arrangement for artificial respiration is shown in Figure 8. The rat's head was firmly held by attaching an adjustable hand vise to the electrode assembly that was cemented to the skull. The method of respiration and infusion was as described in Experiment 1 with the following exception. Unlike the first experiment in which the PIP was initially set at 18-20 cms of water, here PIP was adjusted to 15 cms and maintained at this value until appropriate electrodes were applied. Adjustments were made after this time to bring heart rate into the 400-480 bpm range.

A sterile ophthalmic solution, Isopto tears (0.5% with respect to NaCl) was placed on the rat's eyes to keep them moist throughout the experimental session. Continuous intra-oral suction was accomplished by placing a small section of tubing (PE 200) under the tongue. Low level suction was provided by a Gompco vacuum pump to prevent the build up of fluids which might otherwise be blown down the trachea by the force of positive pressure ventilation.

The animal rested on a disposable diaper on a lucite platform that was warmed by an electric heating pad. After application of the appropriate electrodes, the disposable diaper was brought up and secured across the rat's back. A black lucite panel was attached to the front of the platform to

FIGURE 8 - Preparation for studying operant heart-rate conditioning in the paralyzed rat.



limit the visual field. The rat and the platform were then transferred into a ventilated chamber.

Electrophysiological Recording

All animals were implanted with subdermal EKG electrodes and handled as described in Experiment 2. Electrodermal electrodes were preselected and prepared as in Experiment 1. These were referenced to a site prepared on the right upper thigh.

Chest excursions were measured by a 9" mercury strain gauge (.015 ID, .05 OD) that was wrapped around the animal's chest and connected to a Beckman strain gauge coupler (#9875B). Chest circumference was maintained at a constant value throughout the session in the following way.

Amplifier sensitivity was adjusted to provide a pen deflection of at least 15 mm and not more than 25 mm on each respiratory cycle. The pen deflection at the beginning of the operant-level period was designated as the baseline chest circumference. If at anytime this value either increased or decreased by 2 mms, the peak inspiratory pressure of the respirator was adjusted in steps of 0.5 cms of water, until the original value was reinstated. This procedure was applied to all experimental and yoked control animals, throughout operant level, conditioning and extinction.

Rectal temperature was recorded from a thermister probe (Yellow Springs Instrument Co. #423) that was coated with Vaseline and inserted 6 cms into the rectum. The rectum was evacuated manually before doing this. Throughout the session adjustments were made to the heating pad to prevent the subject from becoming too cool. Initially, the heating pad was set at medium for all rats. If rectal temperature fell below 36.5°C, then the pad was turned to high. If temperature then rose to 37.5°C, the pad was returned to the medium

setting. Both experimental and yoked controls were treated in this manner.

Conditioning Procedure

The animals were assigned to groups on the basis of a strict alternation of order. The first animal was trained to increase its heart rate, the second animal was trained to decrease its heart rate, and so on.

All rats were run in pairs. After application of the appropriate electrodes the rats were left undisturbed for a 30-minute adaptation period. During adaptation, the PIP was adjusted in an effort to produce a stable heart rate, between 400 and 480 bpm, with 15 to 20 bpm variability. At the end of this period, the rat that met the preselection criteria for at least the final 10 minutes of adaptation became the experimental subject. If an animal failed to meet even one of the preselection criteria, it became a control subject. If neither rat met the preselection criteria, the experimental session was terminated for both subjects. If both animals met the criteria, then the rat whose data were recorded on the polygraph that contained circuits for the detection of the RR interval, became the experimental subject. After allocation to appropriate groups, the rats remained undisturbed for a 30-minute operant level period.

Conditioning was then carried out for 90 minutes using the shaping procedure described in Experiment 1, with the addition of a 200-msecs time-out period. During conditioning the yoked control received the same number and distribution of shocks as its experimental counterpart. The circuitry for administering punishments and the shock electrodes was as described in Experiment 1.

Conditioning was followed by 60 minutes of extinction. At the completion of extinction, shock measurements were verified oscilloscopically, and the rat was sacrificed with an overdose of Nembutal.

Results

Conditioning was carried out on 31 rats. Eight animals were trained to increase their heart rates and the same number were trained to decrease their heart rates. In addition, 15 yoked control animals were completed; 7 were yoked to subjects trained to increase, and 8 were yoked to subjects trained to decrease. Seven of the 15 yoked controls satisfied the preselection criteria.

The heart-rate results for both experimental and control subjects are presented in the upper panel of Figure 9. Heart rate was scored from the cardiotachometer channel of the polygraph at 15-minute intervals throughout operant level, conditioning and extinction. Inspection of Figure 9 indicates that although all groups increased their heart rates when shock was introduced, no group was reliably different from any other during the course of punishment training or extinction. Heart-rate change scores were computed for each subject by subtracting the heart rate during the first 15 minutes of extinction from the heart rate during the final 15 minutes of operant level. The difference between the bidirectional groups on this measure was not reliable ($t = -1.66$, $p > .05$) and was actually in the wrong direction.

Although attempts were made to produce lower heart-rate baselines by reducing the PIP in this study, the initial heart rates were still substantially elevated and not reliably different from heart rates observed 30 minutes after curarization in Experiment 2. However, all groups increased their heart rate by more than 10 bpm following the introduction of shock. This finding presents a contrast to the results of Experiment 1, where rats paralyzed by d-tubocurarine failed to show an accelerative unconditioned response to the introduction of shock. This might reflect an increased responsiveness of

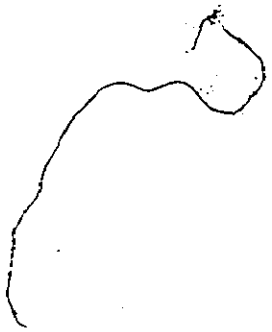
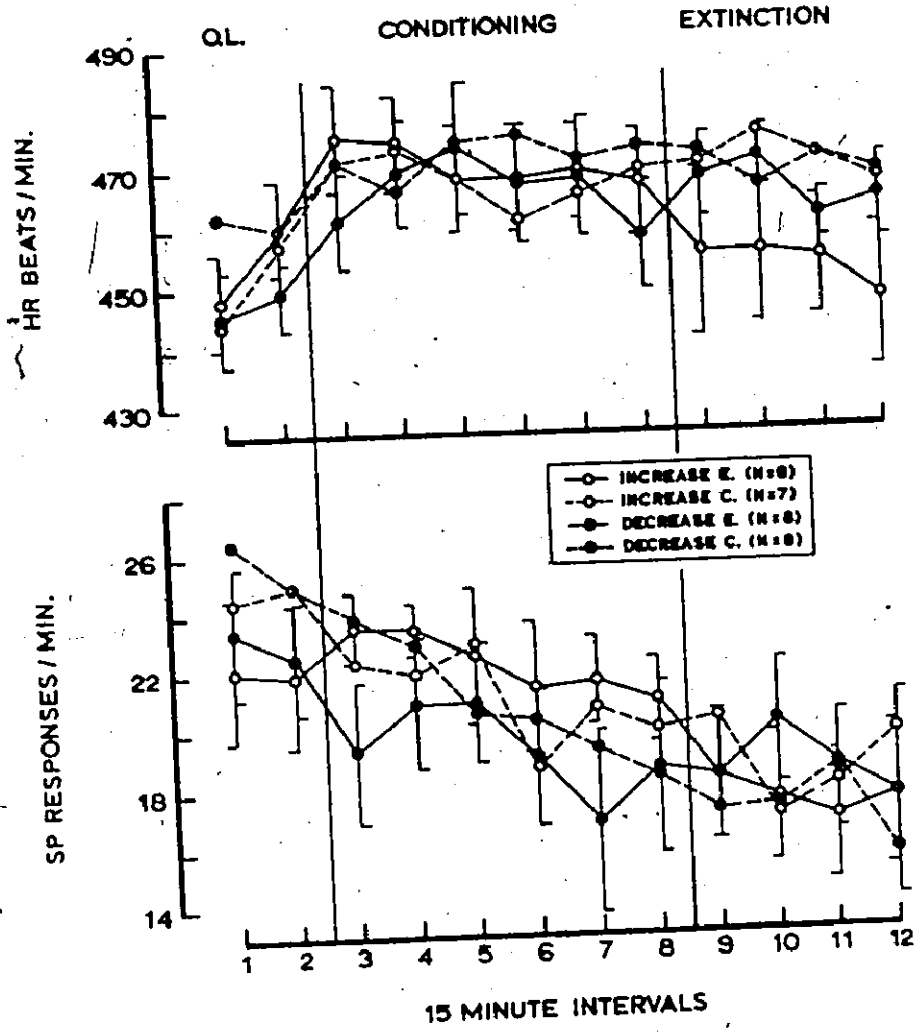


FIGURE 9 - Effect of punishing long and short RR intervals on heart rate (upper panel) and electrodermal activity (lower panel). Rats were paralyzed by succinylcholine.


O.L.
CONDITIONING
EXTINCTION
490
470
450
430
HR BEATS / MIN.
INCREASE E. (N=8)
INCREASE C. (N=7)
DECREASE E. (N=8)
DECREASE C. (N=8)
26
22
18
14
SP RESPONSES / MIN.
1
2
3
4
5
6
7
8
9
10
11
12
15 MINUTE INTERVALS

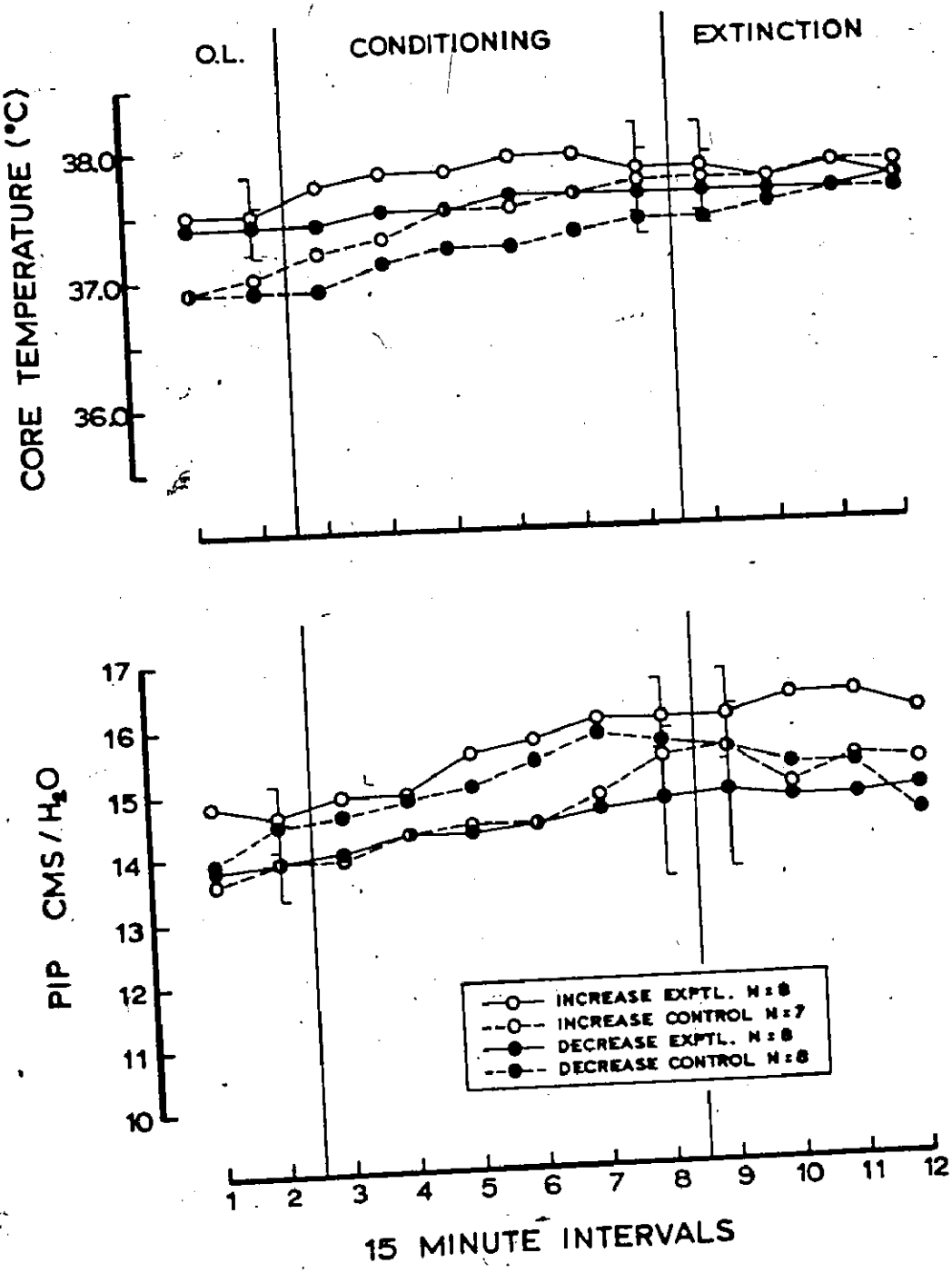
rats in the present study caused by the reduction in PIP, or by the use of succinylcholine. However, it should also be noted that HR baselines were already increasing during the operant-level period for most subjects. For this reason, the extent to which the heart-rate acceleration reflected a UCR to shock rather than a progressively increasing heart-rate baseline is unclear.

The effect of punishing long or short RR intervals on skin-potential responding is depicted in the lower panel of Figure 9. The skin-potential measure is the frequency of phasic responses that occurred 60 seconds prior to the heart-rate measure. Clearly, there was no effect of heart-rate training on skin-potential responding. All groups showed a decline in response frequency throughout the session.

The rectal temperature of both experimental groups increased slightly over time, as presented in Figure 10. However, inspection of the standard errors in this figure shows that there was no reliable difference between the groups throughout training.

The PIPs administered to both experimental and control groups are presented in the lower panel of Figure 10. The PIP adjustments which were made throughout the session to maintain chest excursions constant, produced a PIP at the end of extinction that was significantly higher than the PIP at the beginning of the operant-level period. The increase was apparent and statistically reliable for rats trained to raise ($t = 2.2$, $p < .05$) and lower ($t = 2.6$, $p < .025$) their heart rates. However, the experimental groups did not differ significantly from each other with respect to the number of changes in PIP ($t < 1$) or to the actual PIP at any point during the experiment ($t_{max} = 1.8$, $p > .1$).

FIGURE 10 - Changes in rectal (core) temperature (upper panel) and PIP (lower panel) throughout punishment training.


O.L.
CONDITIONING
EXTINCTION
CORE TEMPERATURE (°C)
38.0
37.0
36.0
PIP CMS / H_2O
17
16
15
14
13
12
11
10
INCREASE EXPTL. N=8
INCREASE CONTROL N=7
DECREASE EXPTL. N=8
DECREASE CONTROL N=8
1 2 3 4 5 6 7 8 9 10 11 12
15 MINUTE INTERVALS

The number of shocks received during each conditioning period is portrayed in the upper panel of Figure 11. There was a tendency for animals trained to decrease their heart rates to receive more shocks than animals trained to increase, but this difference was not statistically reliable ($t < 1$). The increase group received shock for 9.3% of its response distribution, and the decrease group was punished for 9.9% of its response distribution, as averaged over the six conditioning periods.

The maximum interval between shocks, on the other hand, is shown in the lower panel of Figure 11. The measure depicted is the longest interval, measured in cms from the oscillographic record, that was free from shock during each 5 minutes of conditioning. These measurements were combined over 15-minute periods to be comparable to the other analyses. Inspection of this figure reveals that animals trained to decrease their heart rates tended to receive their shocks in bursts, whereas the shocks administered to the increase group were more evenly spaced over time. However, the discrepency between the bidirectional groups in the pattern of shock they received was not reflected in their heart rates, as these did not differ reliably from each other throughout the course of punishment training.

Figure 12 illustrates the relationship between heart-rate change scores computed as described previously and shock density. Despite the fact that a time-out period was employed in the present study as a means of decreasing shock frequency, the punishment densities were not substantially different from those of Experiment 1. However, the range of densities was reduced by this procedure, suggesting greater uniformity was achieved with respect to this variable in the present experiment. An additional finding was that the regressions observed for each group were parallel in the present study, rather than crossed as they were in Experiment 1.

FIGURE 11 – Shock frequency (upper panel) and inter-shock interval (lower panel) throughout conditioning for each bidirectional group.

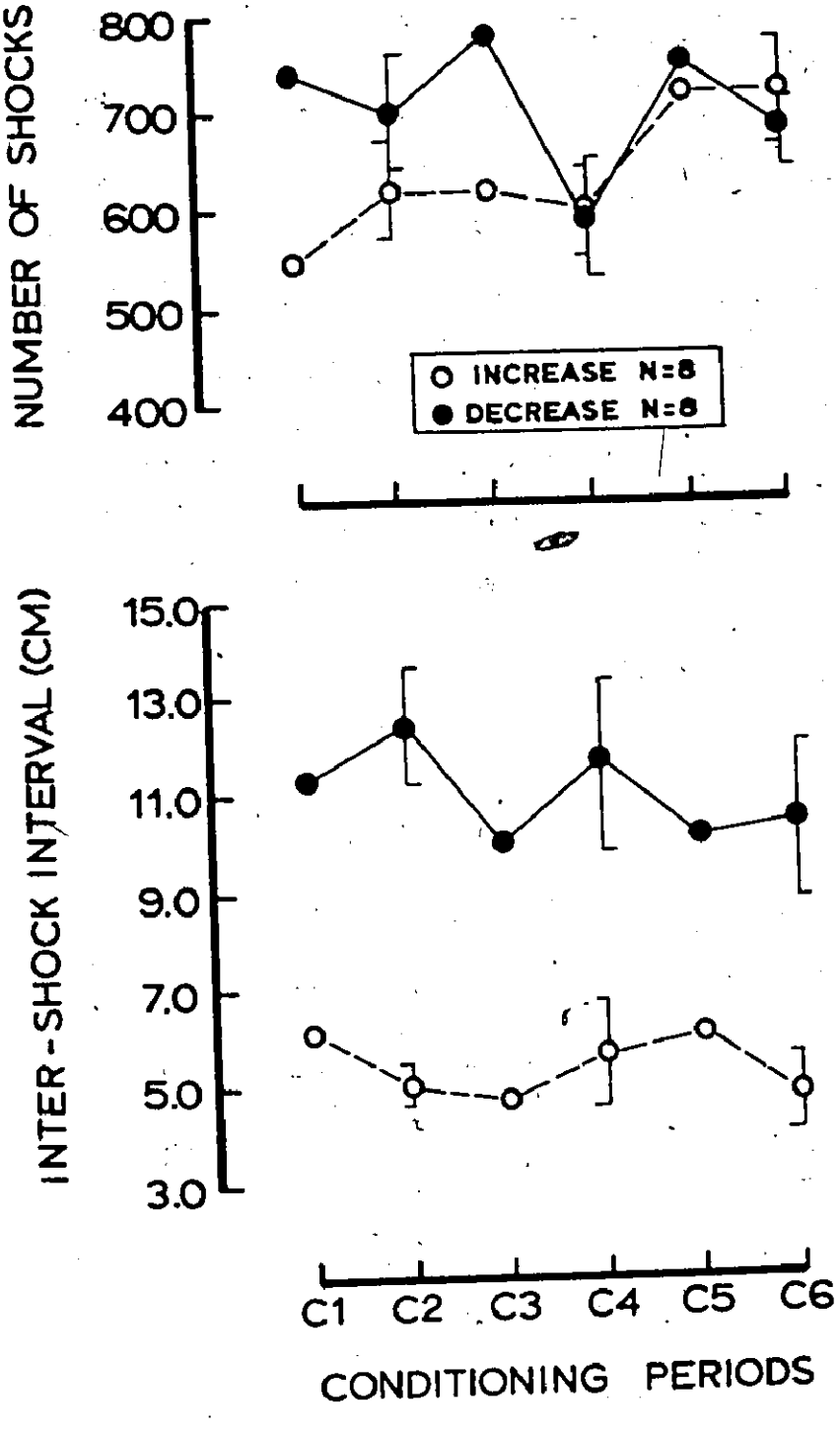
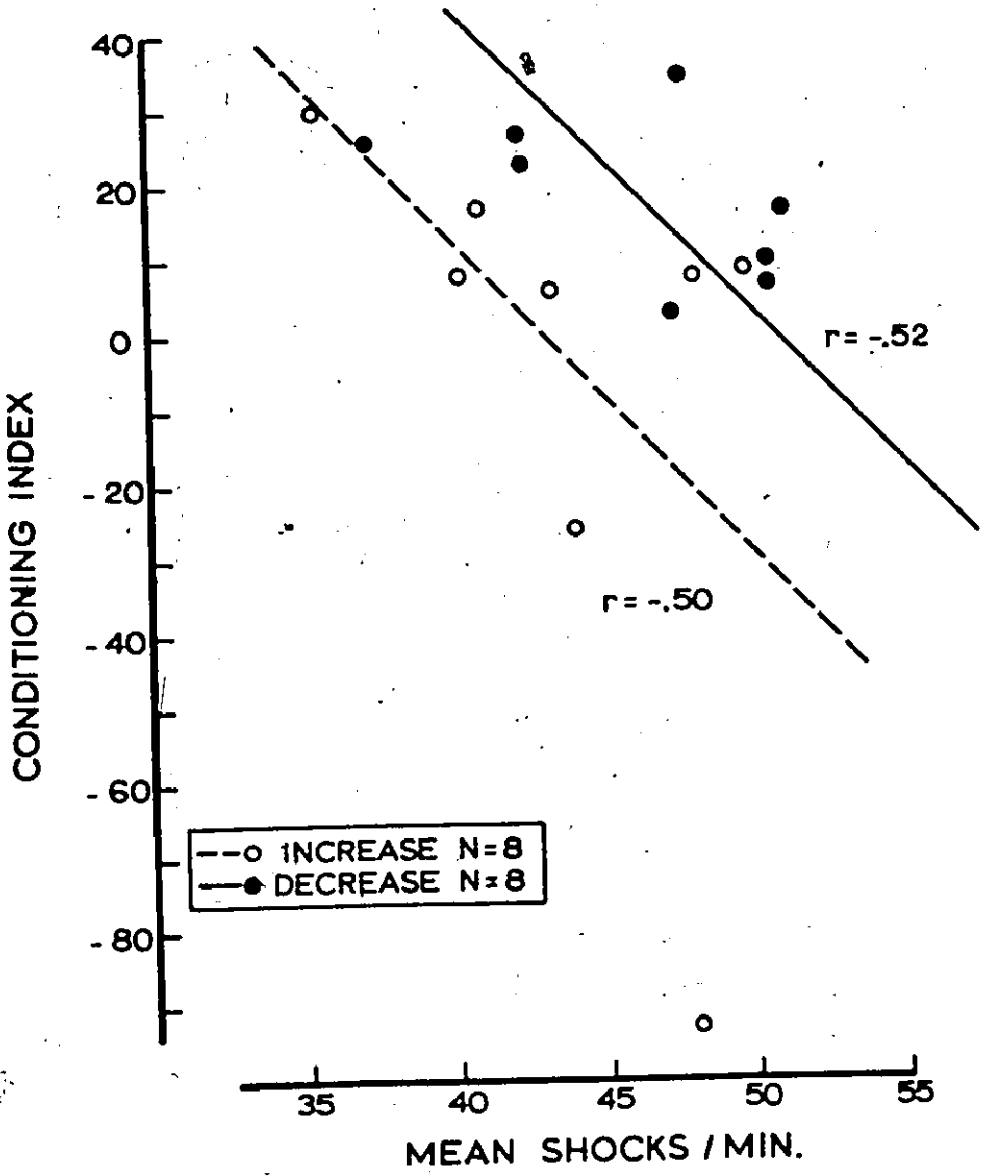

NUMBER OF SHOCKS
800
700
600
500
400
O INCREASE N=8
● DECREASE N=8
INTER-SHOCK INTERVAL (CM)
15.0
13.0
11.0
9.0
7.0
5.0
3.0
C1
C2
C3
C4
C5
C6
CONDITIONING PERIODS

FIGURE 12 – Changes in heart rate during operant conditioning as a function of shock density for each bidirectional group.


40
20
0
- 20
- 40
- 60
- 80
CONDITIONING INDEX
r = -.52
r = -.50
--o INCREASE N=8
—● DECREASE N=8
35
40
45
50
55
MEAN SHOCKS / MIN.

Discussion

Clearly, there was no evidence for the operant conditioning of bidirectional heart-rate differences in the present study. Rats punished for long RR intervals failed to show reliably higher rates at the end of conditioning than animals that were punished for short RR intervals.

It was apparent that heart-rate baselines were not substantially lower in this study, even though the overall PIPs were somewhat decreased from previous experiments. However, the trend of baseline changes was different. The heart-rate baselines in the present experiment tended to increase throughout the session whereas baselines in Experiments 1 and 2 showed a progressive decline over time. This change was probably the result of PIP adjustments that were made to maintain chest excursions at a constant value.

Hahn (1970) indicated that he considered a heart rate in excess of 440 bpm to reflect hyperventilation. All groups in this study displayed operant-level heart rates in excess of this value. These heart rates are characteristic of rats with a decreased pCO_2 (DiCara, 1970; Hahn et al. in press) and suggest that the subjects were hyperventilated. It is possible that evidence for operant conditioning might have been obtained had PIP been lowered still further to prevent hyperventilation.

Brener et al. (1974) reported that chest excursions diminished systematically throughout paralysis when PIP remained constant. This present experiment employed a procedure recommended by Brener to counteract the decrease in chest excursions by increasing the PIP throughout the session. The fact that the PIP required to maintain chest circumference constant was reliable higher at the completion of the experiment than the initial PIP indicates that chest excursions would have diminished over the

course of prolongued paralysis in the present study, had not countermeasures been taken.

One of the reasons that succinylcholine was used for this experiment was that, in Experiment 2, all rats that were paralyzed with this drug displayed intact and stable electrodermal responding. Furthermore, the findings of Experiment 1 suggested that the presence or absence of skin-potential responding was an indicator of conditioning success. All rats in the present study displayed intact electrodermal records. However, the bidirectional groups still failed to show heart-rate changes in the rewarded direction by the end of punishment training.

The results of Experiment 1 also suggested that low shock densities might be important determinants of conditioning. In that study, the subjects that showed the largest heart-rate changes in the rewarded direction were those subjects that received the lowest shock densities. The relationship of shock density to performance was quite different in the present experiment. Although the range of densities was less than in Experiment 1, there was no evidence that the animals receiving the lower shock densities performed differently than other subjects.

CHAPTER FIVE

EXPERIMENT 4

This chapter reports another attempt to operantly condition heart rate in the paralyzed rat. This experiment differs from the previous one in that further efforts were undertaken to avoid hyperventilation, which might prevent operant conditioning from taking place.

Two procedural changes were undertaken to reduce baseline heart rates into the 380-430 bpm range. First, the previous experiment had defined a lower limit for PIP of 12 cms of water, as one of the requirements for the selection of an experimental subject. This limitation was abandoned and no minimum value for PIP was required for the present study. The second procedural change was that the adaptation period was extended to 60 minutes to permit stabilization of a lower heart rate.

Method

Subjects

The subjects for the present experiment were 40 male hooded rats, bred from Quebec Breeding Farms stock and ranging in weight from 320 to 470 gms. Twenty animals were assigned to the experimental group and the remaining 20 were yoked controls. Four pairs of animals (8 subjects) were discarded because the compensatory PIP adjustments initiated by the experimenter to hold chest excursions constant were insufficient to keep the experimental subject alive. In addition, one yoked control subject died during conditioning.

Procedure

The procedure was as described in Experiment 3 with the following exceptions. In order to produce lower heart rates in this study, the PIP was initially set at 12 cms of water. Next, all animals were permitted 60 minutes of adaptation during which the PIP was reduced to bring heart rate into the 380-430 bpm range. Finally, subjects were allocated to increase or decrease groups by the flip of a coin after all respiratory adjustments had been made. This was to ensure that the assignment of subjects to bidirectional groups was independent of current heart-rate trends.

The preselection criteria for choosing the experimental subject differed slightly from those of the previous study. The requirements of the present experiment were a rectal temperature of greater than 35°C, the presence of electrodermal responding, and a heart rate between 380-430 bpm. The criteria for altering the heating pad settings of both experimental and yoked control animals were also modified for the present study. In Experiment 3, the heating pad was adjusted if the animal's temperature fell below an acceptable value, but nothing was done if the animal became too warm. The procedure for dealing with low temperatures in the present study remained as described in Experiment 3. However, if rectal temperature reached 38.5°C, then the heating pad was turned off until temperature returned to 37.5°C when the pad was turned again to the medium setting.

The shaping procedure and shock parameters were similar to those used in Experiment 1. In addition, the method used to complete the IRT distributions and the rules for altering criteria (described on Page 13) remained the same. However, shock density was reduced in this study by

lowering the proportion of the animals' IRT distribution that was punished from 10 to between 5-7%. If the animal altered its heart rate in the rewarded direction, the criterion was shifted so that once again 5-7% of the response distribution was punished. If the rat failed to reach criterion on more than two successive distributions, then criterion was relaxed. The experimenter attempted to shock only 5-7% of the subject's response distribution and to maintain temporal density at one shock every 1-3 secs.

Electrophysiological Recording

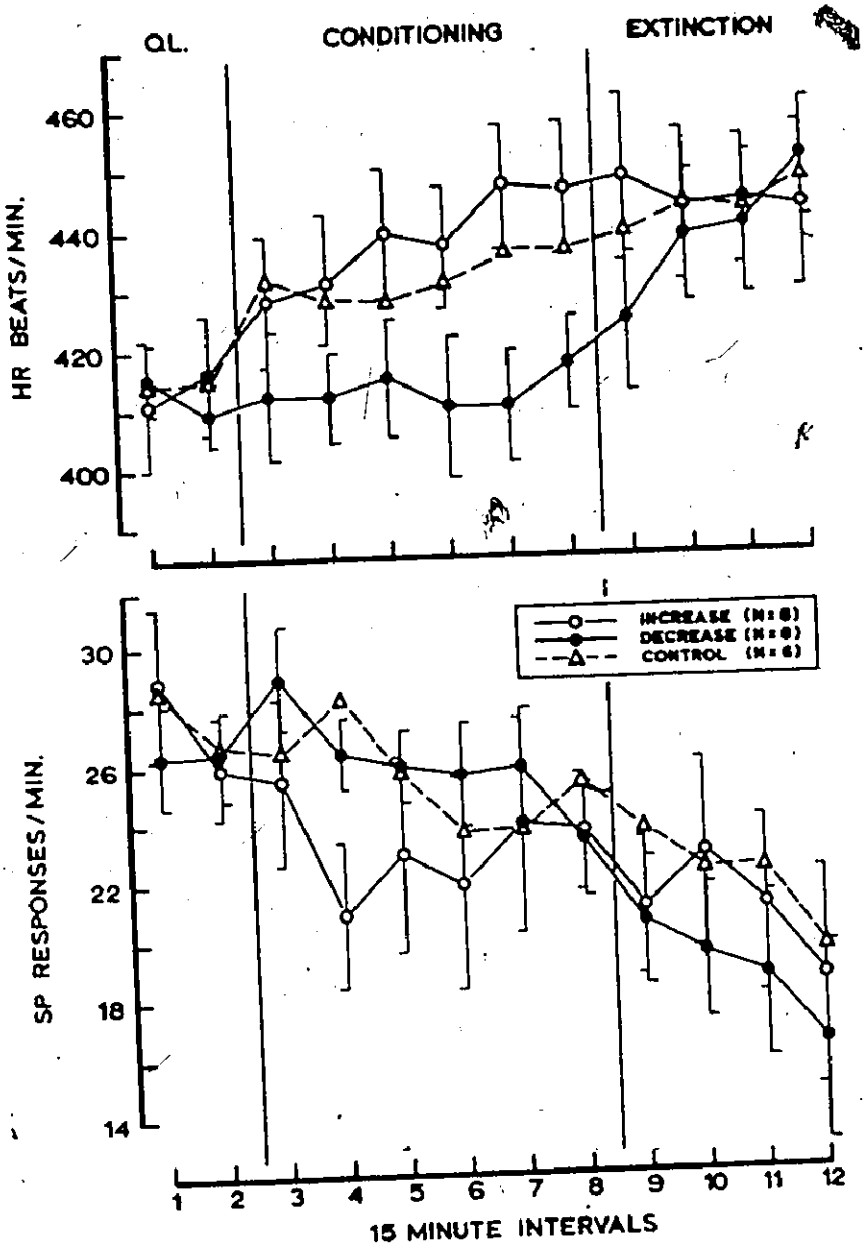
The electrophysiological recording remained as described in Experiment 3.

Results

Conditioning was carried out on 31 rats. Eight animals were trained to increase and 8 animals were trained to decrease their heart rates. Fifteen yoked control subjects also completed training. Seven were yoked to increase subjects and the remainder were yoked to decrease animals. In order to make the data more interpretable, only those yoked controls that met the preselection criteria were included in the analyses. A total of 22 of the 31 experimental and control subjects met the preselection criteria for acceptance.

The effect of punishment training on heart rate is portrayed in the upper panel of Figure 13. Data from the increase and decrease yoked control subjects have been combined in this figure to increase the sample size. Inspection of Figure 13 reveals that although there was no difference between the bidirectional groups during the operant-level period, by the end

FIGURE 13 - Effect of punishing long or short RR intervals on heart rate (upper panel) and electrodermal activity (lower panel).


O.L.
CONDITIONING
EXTINCTION
HR BEATS/MIN.
460
440
420
400
INCREASE (N=6)
DECREASE (N=6)
CONTROL (N=6)
SP RESPONSES/MIN.
30
26
22
18
14
1
2
3
4
5
6
7
8
9
10
11
12
15 MINUTE INTERVALS

of conditioning rats that were trained to increase displayed substantially higher heart rates than those trained to decrease. The difference between the bidirectional groups was in the expected direction and was statistically reliable during the 6th, and 7th 15-minute intervals of conditioning ($t_{min} = 2.14$, $p < .05$). Differences between the bidirectional groups and yoked controls were not statistically reliable although they were in the expected direction..

Table 2 presents heart-rate change scores for individual rats. These were computed at both 5 and 15 minutes into extinction. Inspection of Table 2 reveals that all animals trained to increase their heart rates did so reliably, while rats trained to decrease failed to actually lower their heart rates below the operant-level value. The effect in the increase group was present and statistically reliable at both 5 ($t = 3.03$, $p < .01$) and 15 minutes ($t = 4.19$, $p < .005$) after the shock was terminated, whereas the decrease result was not significant at either point (5 minutes, $t < 1$; 15 minutes, $t = 1.64$, $.05 < p < .10$). As can be seen in the left panel of Table 2, the difference between the bidirectional groups was statistically significant ($t = 2.2$, $p < .025$) at 5 minutes into extinction. However, the right panel of Table 2 reveals that the bidirectional difference had diminished after 15 minutes of extinction and was not statistically reliable at this point ($t = 1.11$, $p > .10$).

The effect of punishment training was evident during extinction as well, where the bidirectional difference diminished reliably as extinction progressed. A 2 x 4 repeated measures analysis of variance was completed with direction of training and conditioning period as the between and within variables respectively. This analysis revealed that the interaction of

TABLE 2 – The effect of punishing the RR interval in rats paralyzed by succinylcholine. The HR measure is obtained by subtracting HR during the first 5 min (left panel) or 15 min (right panel) of extinction from heart rate during operant level for each rat.

Table 2

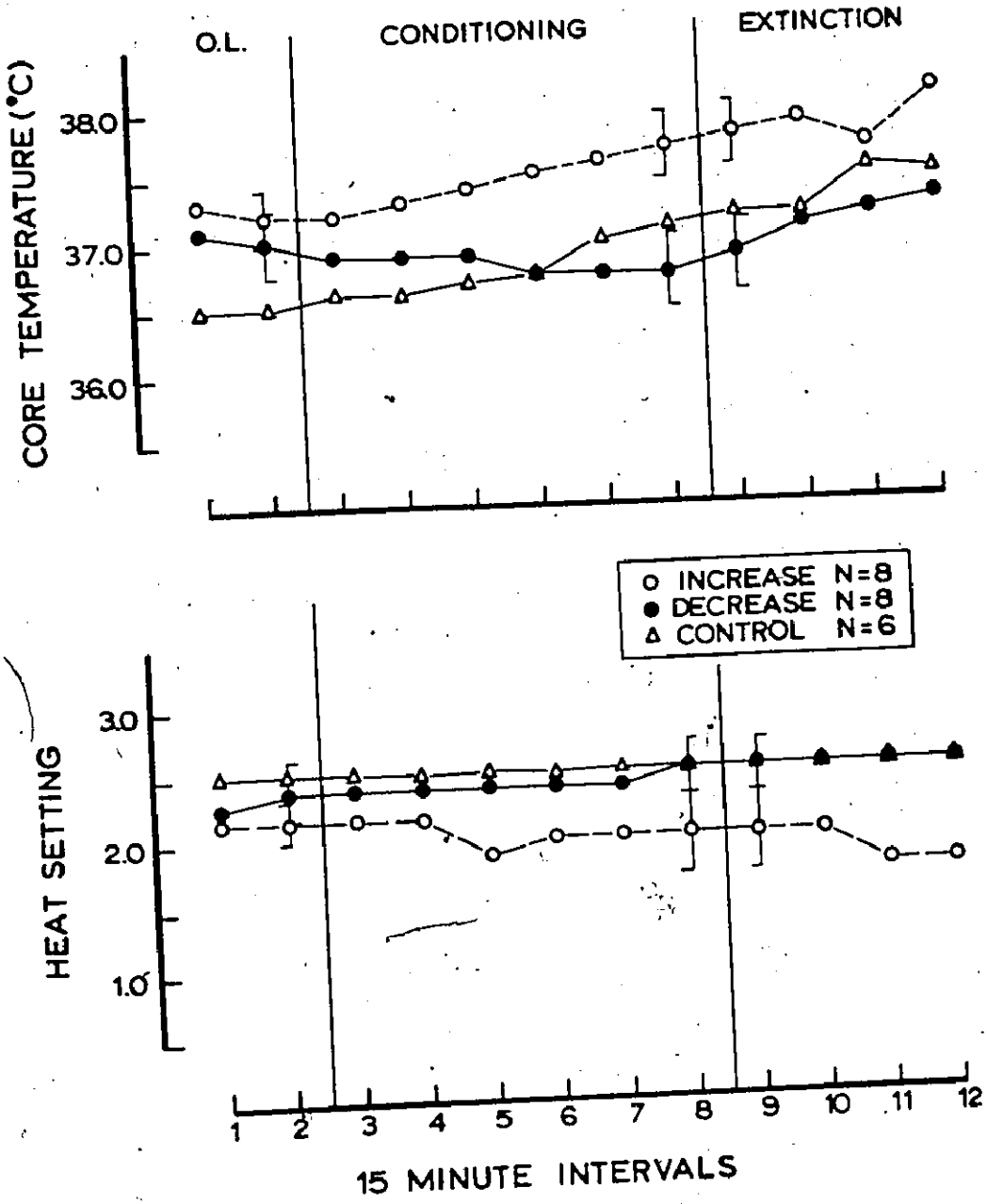
Measurement At 5 Minutes into Extinction		Measurement At 15 Minutes into Extinction	
Increase	Decrease	Increase	Decrease
+ 3	-45	+ 3	-23
+16	- 3	- 2	- 9
+40	+27	+57	+26
+42	- 8	+21	+12
+39	+34	+43	+56
+18	+11	+20	-21
+43	-21	+53	+52
+45	+42	+57	
M 30.8	4.62	31.5	16.0
t 3.03	.16	4.19	1.64
p < .01	ns	< .005	ns
t = 2.2		t = 1.11	
p < .025		ns	

direction of training and conditioning period was statistically reliable ($F = 3.91$, $df = 3,42$, $p < .05$).

The effect of punishment training on skin-potential responding is depicted in the lower panel of Figure 13. The measure is the frequency of phasic skin potential responses that occurred 60 seconds prior to the heart-rate measure. Perusal of this figure indicates that bidirectional heart-rate training had no systematic effect on skin-potential responses frequency. All groups showed a gradual decline throughout the study. This decrease in frequency between the first and last 15-minute conditioning periods was reliable for both the increase ($t = 2.29$, $p > .05$) and the decrease ($t = 2.62$, $p < .025$) groups. In addition, all animals in this experiment displayed electrodermal activity.

An attempt was made throughout the experiment to maintain rectal temperature within a moderate range by adjusting an electric heating pad. The temperature of the bidirectional groups and yoked controls is portrayed in the upper panel of Figure 14. This figure reveals that although there was little difference between the increase and decrease groups during the operant level period, by the end of conditioning, a bidirectional difference had developed that was reliable and in the same direction as the heart-rate effect ($t = 2.38$, $p < .025$). The lower panel of Figure 14 indicates that this bidirectional difference cannot be attributed to differential heating pad settings, as the decrease group experienced a higher setting and still exhibited lower temperatures than did the increase group. Furthermore, the difference between the groups in heat settings was not reliable. For these reasons, it appears that the temperature differences were the result of the heart-rate changes, rather than of differential heating pad adjustments. The relationship between changes in heart rate and

FIGURE 14 - Changes in rectal (core) temperature (upper panel) and heat setting of the heating pad (lower panel) throughout punishment training. The numbers on the ordinate of the lower panel represent settings as follows: 1.0 - low 2.0 - medium, 3.0 - high.


O.L.
CONDITIONING
EXTINCTION
CORE TEMPERATURE (°C)
38.0
37.0
36.0
INCREASE N=8
DECREASE N=8
CONTROL N=6
HEAT SETTING
3.0
2.0
1.0
1 2 3 4 5 6 7 8 9 10 11 12
15 MINUTE INTERVALS

temperature for experimental subjects is plotted in Figure 15. Inspection of this figure indicates that changes in these two variables over the course of training were correlated (r = +.65).

PIP was adjusted for all subjects at various points throughout the study, to maintain chest excursions constant. The upper panel of Figure 16 portrays the PIP administered to the bidirectional and control groups throughout the course of punishment training. Although heart rates of rats trained to increase differed reliably from those trained to decrease, the groups did not differ at any point throughout the session with respect to PIP (t_{max} = 1.19, p > .10). The PIP increased slightly throughout the experiment in all groups. Furthermore, there was no reliable difference in the mean number of adjustments made to the bidirectional groups. The increase animals received 4.8 adjustments, whereas the decrease group was adjusted 4.2 times per subject (t < 1).

The criterion for adjusting the PIP was a change in pen deflection on the polygraph of 2 mm in either direction. If this change occurred, then the experimenter increased or decreased the PIP in steps of 0.5 cms of water, until the original chest circumference value was reinstated. The lower panel of Figure 16 portrays the change in chest circumference throughout the experimental session. The horizontal lines demarcate the increase or decrease in chest circumference required for an adjustment in PIP by the experimenter. Inspection of this figure indicates that adjustments made to the PIP in the present experiment produced relatively stable chest excursions over the course of punishment training.

Analysis of the shock records revealed that the increase groups was punished for 7.2% of its response distribution whereas the decrease group

FIGURE 15 – Changes in heart rate as a function of changes in rectal (core) temperature. Temperature during the last 15 min of operant level has been subtracted from temperature during the first 15 min of extinction. The heart rate measure was computed in the same way.

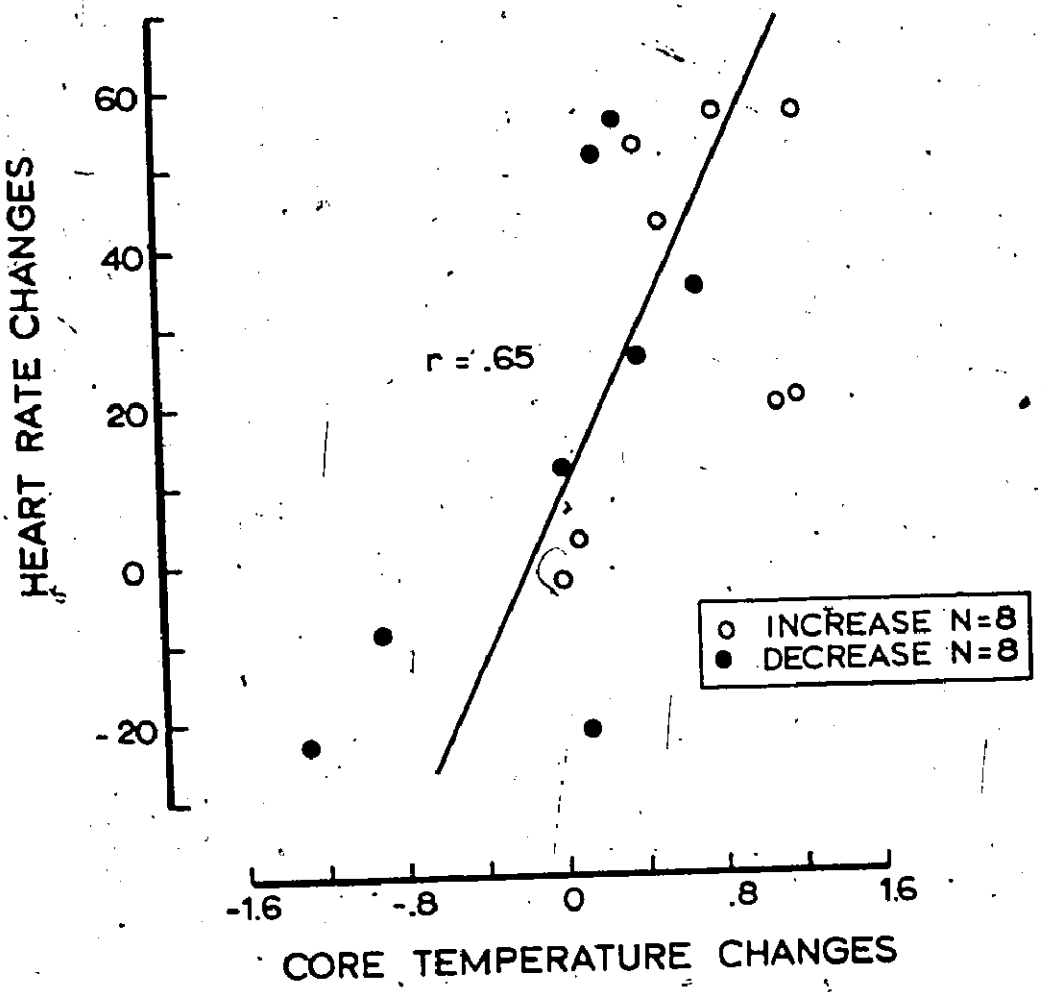
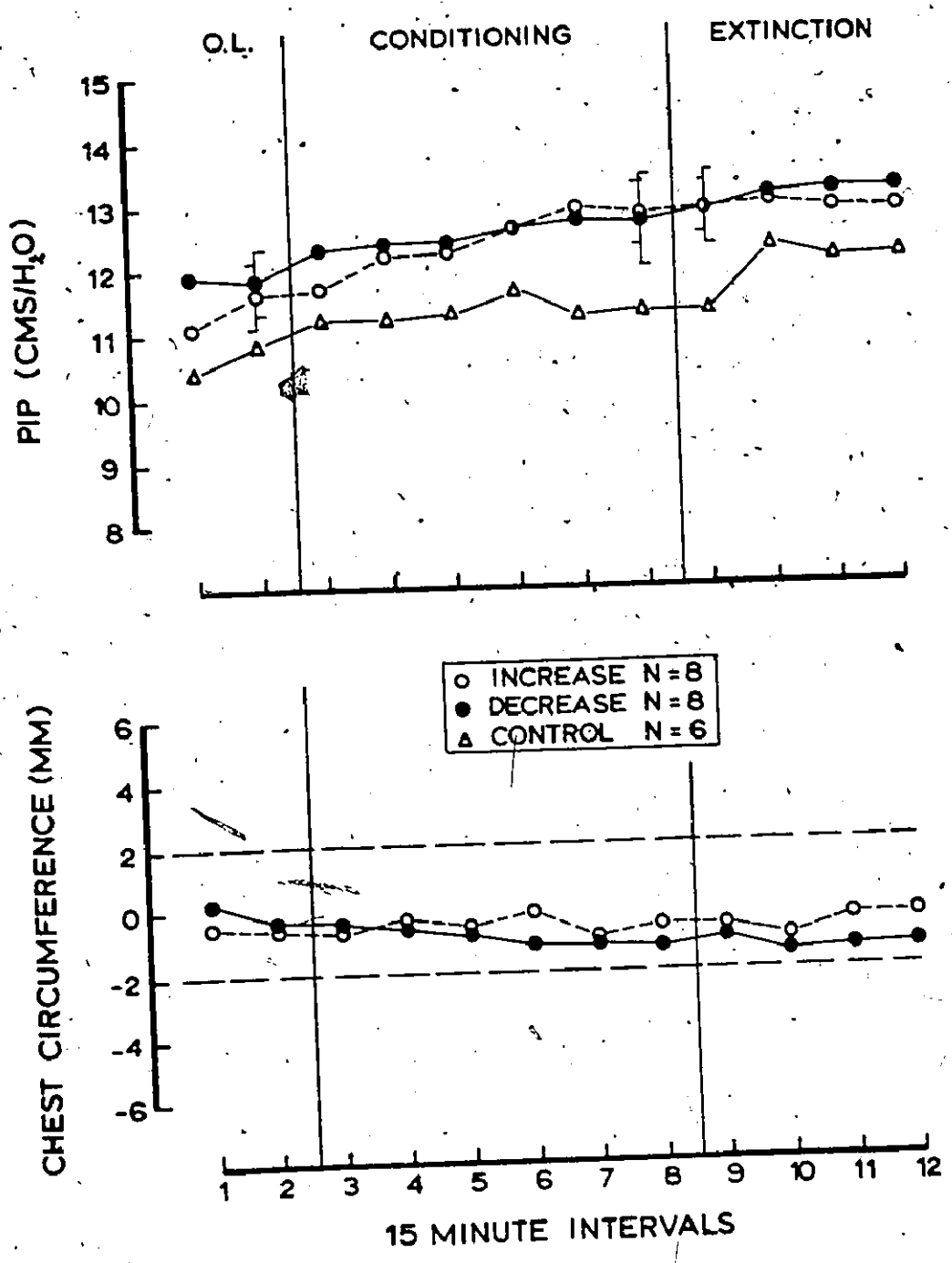

HEART RATE CHANGES
60
40
20
0
-20
r = .65
INCREASE N=8
DECREASE N=8
-1.6
-.8
0
.8
1.6
CORE TEMPERATURE CHANGES

FIGURE 16 – Changes in PIP (upper panel) and chest circumference (lower panel) throughout punishment training.


O.L.
CONDITIONING
EXTINCTION
PIP (CMS/H2O)
15
14
13
12
11
10
9
8
INCREASE N=8
DECREASE N=8
CONTROL N=6
CHEST CIRCUMFERENCE (MM)
6
4
2
0
-2
-4
-6
1
2
3
4
5
6
7
8
9
10
11
12
15 MINUTE INTERVALS

received shock for 6.6% of its response distribution. This difference was not statistically reliable ($t = 1.18$, $p > .10$). However, there was a reliable difference in the total number of shocks received by the bidirectional groups. The relevant data are shown in the upper panel of Figure 17, where it is apparent that animals trained to raise their heart rates received more shocks than rats trained to lower their heart rates. Although the difference in the number of shocks received by each group was small, it was statistically reliable when considered for training as a whole ($t = 2.3$, $p < .05$). This difference is an inevitable consequence of shaping procedures that punish exactly the same percentage of responses in groups that develop divergent heart rates.

As in the previous study, differences in the pattern of shock were evaluated by measuring, for consecutive 5-minute periods, the longest interval that was free from shock. Inspection of the lower panel of Figure 17 reveals that subjects trained to decrease their heart rates tended to receive their shocks in bursts, whereas the pattern of punishments administered to the increase group was more uniform. However, this pattern difference was greatest in the early periods of punishment training and had disappeared by the end of conditioning. The overall difference was marginally significant ($t = 1.94$, $p < .1$).

An attempt was also made to determine the form of the unconditoned response to the introduction of shock in the present study. This was accomplished by examining the predominant change in HR during the 10 seconds following the first shock. Responses were classified as either biphasic, accelerative or decelerative. No attempt was made to quantify the magnitude of the unconditioned response. Table 3 depicts the relationship between

FIGURE 17 - Shock frequency (upper panel) and inter-shock interval (lower panel) throughout punishment training.

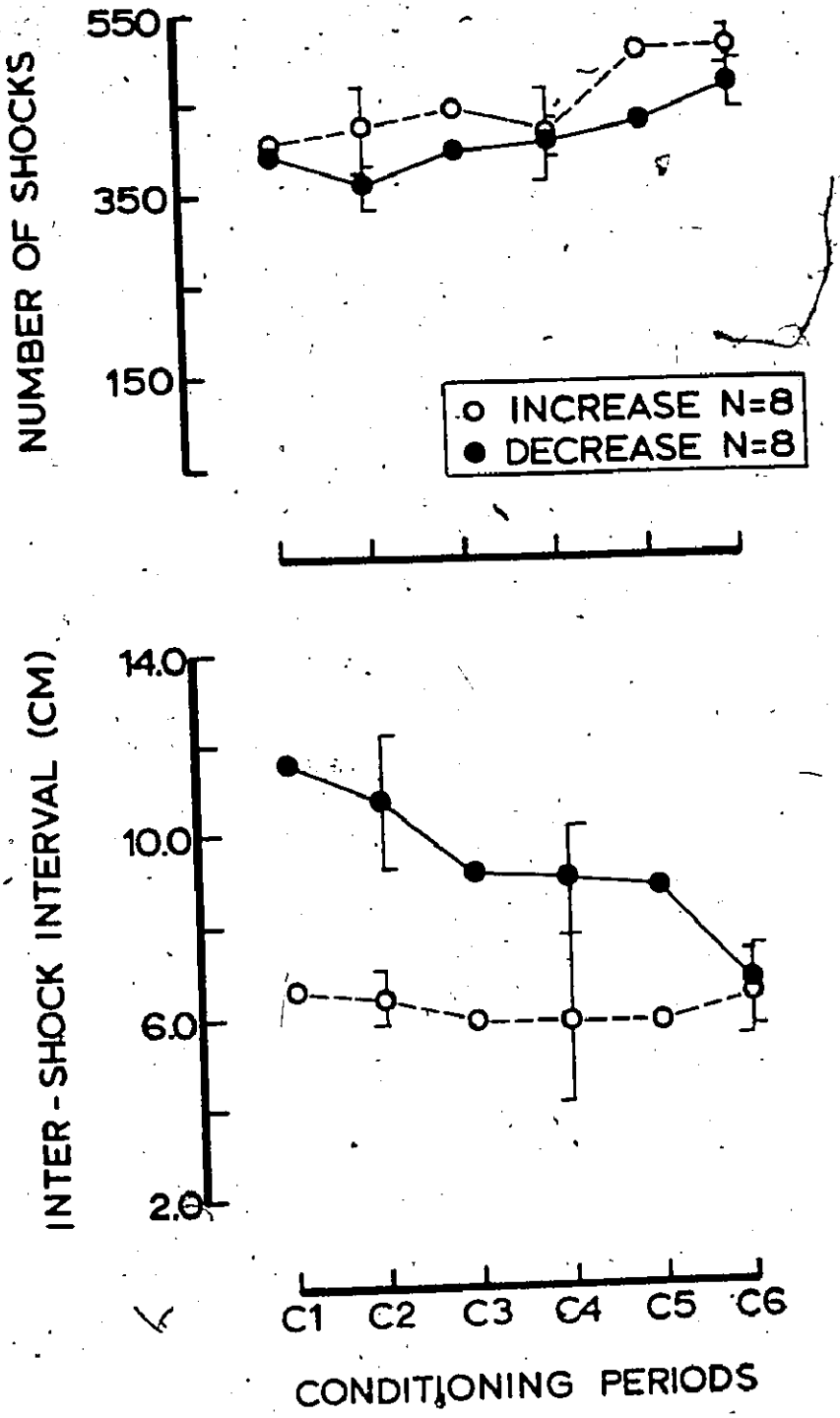

NUMBER OF SHOCKS
550
350
150
o INCREASE N=8
● DECREASE N=8
INTER-SHOCK INTERVAL (CM)
14.0
10.0
6.0
2.0
C1
C2
C3
C4
C5
C6
CONDITIONING PERIODS

TABLE 3 - The direction of the UCR to the introduction of shock for individual rats. The numbers in each cell are the shocks frequencies for each subject during the first 15 min of conditioning.

Table 3

	INCREASE	DECREASE
INCREASE	42.4 30.6 $\bar{x}$ = 36.5	28.1 25.5 $\bar{x}$ = 26.8
BIPHASIC	26.6 29.9 26.6 $\bar{x}$ = 27.7	30.1 27.2 27.4 26.5 25.0 $\bar{x}$ = 27.2
DECREASE	33.7 32.0 26.8 $\bar{x}$ = 30.8	27.4 $\bar{x}$ = 27.4

direction of training and the form of the UCR for each rat. The data presented in the individual cells represent shock density during the first conditioning period. Inspection of Table 3 indicates that the direction of the UCR was not the same in all rats. Subjects displayed either an increase, a decrease or a biphasic response to the introduction of shock. Also, the distribution of these responses was relatively constant across the bidirectional groups.

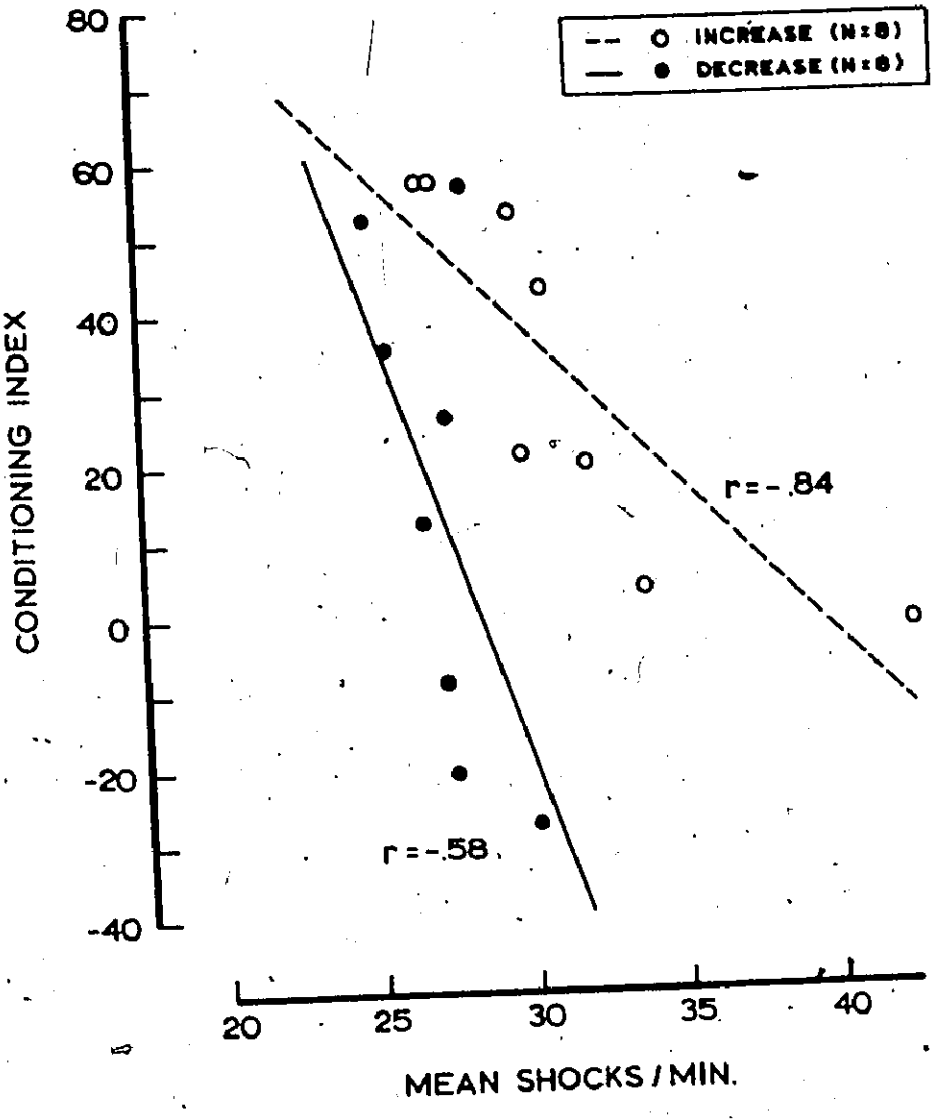
Heart-rate change scores, computed by subtracting heart rate during the final 15 minutes of operant level from heart rate during the first 15 minutes of extinction, are plotted as a function of shock density in Figure 18. The range of shock densities in the present experiment (25-43 shocks per minute) was reduced considerably from those in Experiment 1 (15-90 shocks per minute), indicating more uniformity in this variable. Further, overall shock densities were reduced as compared to Experiments 1 and 3. The mean shock density in the present study was 29.3 shocks per minute, a value that was reliably lower than the densities reported in Experiment 1 ($\underline{M}$ = 53.0; $\underline{p} < .01$) and Experiment 3 ($\underline{M}$ = 44.9; $\underline{p} < .05$).

However, inspection of Figure 18 reveals that the direction of the relationship between heart-rate change and shock density was not different between the bidirectional groups, as was the case in Experiment 1.

Discussion

The results of the present experiment were consistent with the view that operant conditioning had taken place. By the end of training, subjects punished for long RR intervals displayed heart rates that were significantly higher than the heart rates of animals punished for short RR intervals. This bidirectional difference occurred gradually and diminished systematically

FIGURE 18 – Changes in heart rate as a function of shock density.


INCREASE (N=8)
DECREASE (N=8)
80
60
40
20
0
-20
-40
CONDITIONING INDEX
r=-.84
r=-.58
20
25
30
35
40
MEAN SHOCKS / MIN.

over the course of extinction.

Before these data can be interpreted as evidence for operant conditioning, several alternative hypothesis must be considered. The most obvious of these is that the heart-rate changes were produced by differences in the number of shocks administered to the bidirectional groups. Rats trained to raise their heart rates received significantly more shocks than animals trained to lower. However, this interpretation is rendered unlikely on the basis of two additional findings. Attribution of the bidirectional difference in heart rate to the difference in shock frequency requires the assumption that heart rate is an increasing function of shock density. Figure 18 reveals however, that heart-rate changes for both bidirectional groups were a decreasing function of this variable. Secondly, the direction of the unconditioned response was not the same for all rats. Animals displayed either heart-rate accelerations, decelerations or biphasic responses to the introduction of shock. Further, the distribution of these responses across bidirectional groups was relatively uniform. This result is incompatible with a shock frequency interpretation, which would predict that the predominant unconditioned response was a heart-rate acceleration.

Another interpretation suggests that differential adjustments in PIP produced the heart-rate differences. However, at no time during the experiment was there any reliable difference between the PIPs applied to both groups, or in the number of times the PIP was adjusted in each bidirectional group. Alternatively, it could be argued that although there was no difference in actual PIP, the adequacy of respiration parameters differed between the groups. Reliable differences in heart rate might result if the decrease subjects became progressively hypoventilated over time, or if the increase rats became

hyperventilated over the session. However, chest excursions for each bidirectional group remained stable over training with no divergence between them.

A third interpretation of these data suggests that the reliable difference in rectal temperature produced the heart-rate difference. However, changes in heart rate and temperature were highly correlated across subjects in the present study, and persisted despite attempts by the experimenter to compensate by adjusting the heating pad. This suggests that the temperature differences were determined by the heart-rate differences and not vice versa.

In summary, the results of this study indicate that neither PIP nor core temperature adjustments made by the experimenter, nor differences in shock frequency produced the bidirectional changes in heart rate. These findings present the most favourable evidence for operant conditioning of heart rate of any experiments discussed in this thesis. However, it is not clear why bidirectional differences occurred in this study but not the previous one. The two most likely explanations are that hyperventilation was lessened by lowering PIP and the heart-rate baseline, or that shock density was reduced by punishing a smaller proportion of the rat's responses. The question of whether the bidirectional difference observed in the present study can be attributed to operant conditioning is discussed further in the following chapter.

CHAPTER SIX

DISCUSSION AND CONCLUSIONS

Consideration of the learning experiments reported in this thesis presents a mixed picture as to the conditionability of heart rate in the paralyzed rat. Reliable differences in heart rate were obtained in Experiments 1 and 4, whereas subjects in Experiment 3 failed to display bidirectional differences in heart rate by the end of punishment training. The findings of other investigators are mixed as well. Dworkin (1973) reported that he was unable to produce bidirectional heart-rate differences in two experiments that utilized the shock avoidance procedure of the earlier Miller and DiCara studies (DiCara & Miller, 1968a). Middaugh (1971) was also unable to train bidirectional heart-rate differences in an experiment that used ESB as the reinforcer. However, in a subsequent study from the same thesis (Middaugh, 1971), a small but reliable bidirectional change in heart rate was produced, using a different shaping procedure.

One interpretation of these inconsistent findings is that the bidirectional differences in heart rate, reported in earlier experiments, were produced by variables other than the operant contingency. The nature of some of these variables and their possible role in the present studies is considered below.

Non-Contingency Variables

One such variable that received considerable attention in this thesis

was peak inspiratory pressure. This parameter was adjusted throughout training by the experimenter in both Experiments 3 and 4. It is possible therefore, that differential adjustments in PIP to the increase and decrease groups could produce a bidirectional difference in heart rate. However, this problem was anticipated during the planning of these studies, and the rules for PIP adjustments were rigidly defined and executed on the basis of a discrete change in chest excursions. Furthermore, chest circumference was monitored and recorded on a breath-by-breath basis throughout the experiment and analyzed fully at the completion of the study. However, detailed examination of both chest circumference and PIP measures provided no support for an interpretation of the heart-rate changes in terms of differences in peak inspiratory pressure.

Another variable that was manipulated by the experimenter throughout training was the setting on the heating pad. This raises the possibility that the bidirectional difference in heart rate was produced by differential heat settings applied to the bidirectional groups. According to this hypothesis, rats trained to increase may have been heated more warmly than rats trained to decrease their heart rates. This assumes that heart rate is accelerated by warming, as demonstrated in human subjects by Wyss (1974). However, the heating-pad settings received by the bidirectional groups were in a direction that was opposite that predicted by a hypothesis of this nature. This suggests that if anything, the compensatory adjustments to the heating pad attenuated the heart-rate differences. It does not appear then, that the bidirectional differences in heart rate, produced in Experiment 4, can be attributed to the experimenter's manipulation of the setting on the heating pad.

The method of determining the direction of heart-rate training in Experiments 1 and 3 is open to criticism. The direction of training was known to the experimenter while respiratory adjustments were made during the adaptation period. This suggests that the experimenter's expectation concerning the animal's performance during learning may have influenced the adjustments made to PIP during the adaptation period. For this reason, allocation of subjects to bidirectional groups was randomized in Experiment 4, to ensure that direction of training was independent of current heart-rate trends that occurred during the adaptation period. Still, a bidirectional difference in heart rate was reported in Experiment 4. This variable then does not seem to have been responsible for the reliable changes in heart rate reported in that study.

The variables associated with the shaping procedure are more difficult to define and evaluate. The rules determining criterion changes during the shaping procedure were delineated before each study and adhered to as strictly as possible. However, it was frequently necessary to recalculate the punishment criterion more often than once per minute as originally planned, to ensure that the rats were shocked approximately once every two seconds. This raises the possibility that the experimenter's adjustment of punishment criteria may not have been made independently of her expectation with respect to the outcome of conditioning.

Evaluation of the role of possible experimenter biases in the application of the shaping procedure is extremely difficult. It requires as a first step the formulation of an hypothesis about how such a bias might have determined conditioning outcomes. One such possibility that can be evaluated on the basis of the results provided in Experiment 4 is as follows.

This study revealed that the unconditioned response to the introduction of shock was not uniform for all subjects. Some rats showed an increase, some a decrease, and others a biphasic change in heart rate. This raises the possibility that the experimenter might have administered more shocks to an increase rat that displayed an accelerative UCR, than to an increase rat that displayed a decelerative UCR. The reverse may have occurred for rats trained to decrease their heart rates. A bias of this sort could easily have generated a bidirectional difference that was not the result of an operant contingency.

The data presented in Table 3 permit an evaluation of this type of bias. Inspection of the increase group presented in the left panel of Table 3 reveals that animals that showed an accelerative UCR received more shocks than rats that displayed a decelerative UCR ($\underline{M}$ = 36.5 and $\underline{M}$ = 31.0 respectively). Furthermore, this trend was reversed in the decrease groups where rats that showed a decelerative UCR received more shocks than subjects that displayed an accelerative UCR. Unfortunately, the number of rats contributing to this trend is not sufficient to permit a meaningful evaluation of statistical significance. It is relevant to note, however, that the rats that contributed most prominently to this trend did not contribute disproportionately to the bidirectional difference found in Experiment 4.

It should be stressed that the bias evaluated in Table 3 represents only one of a large class of biases that may be operating in a study where the experimenter plays an active role. The problems associated with the shaping procedure are extremely difficult, if not impossible to evaluate in the present experiments. Furthermore, with one exception (Fields, 1970a) shaping procedures reported in other studies (Miller & DiCara, 1967; Slaughter,

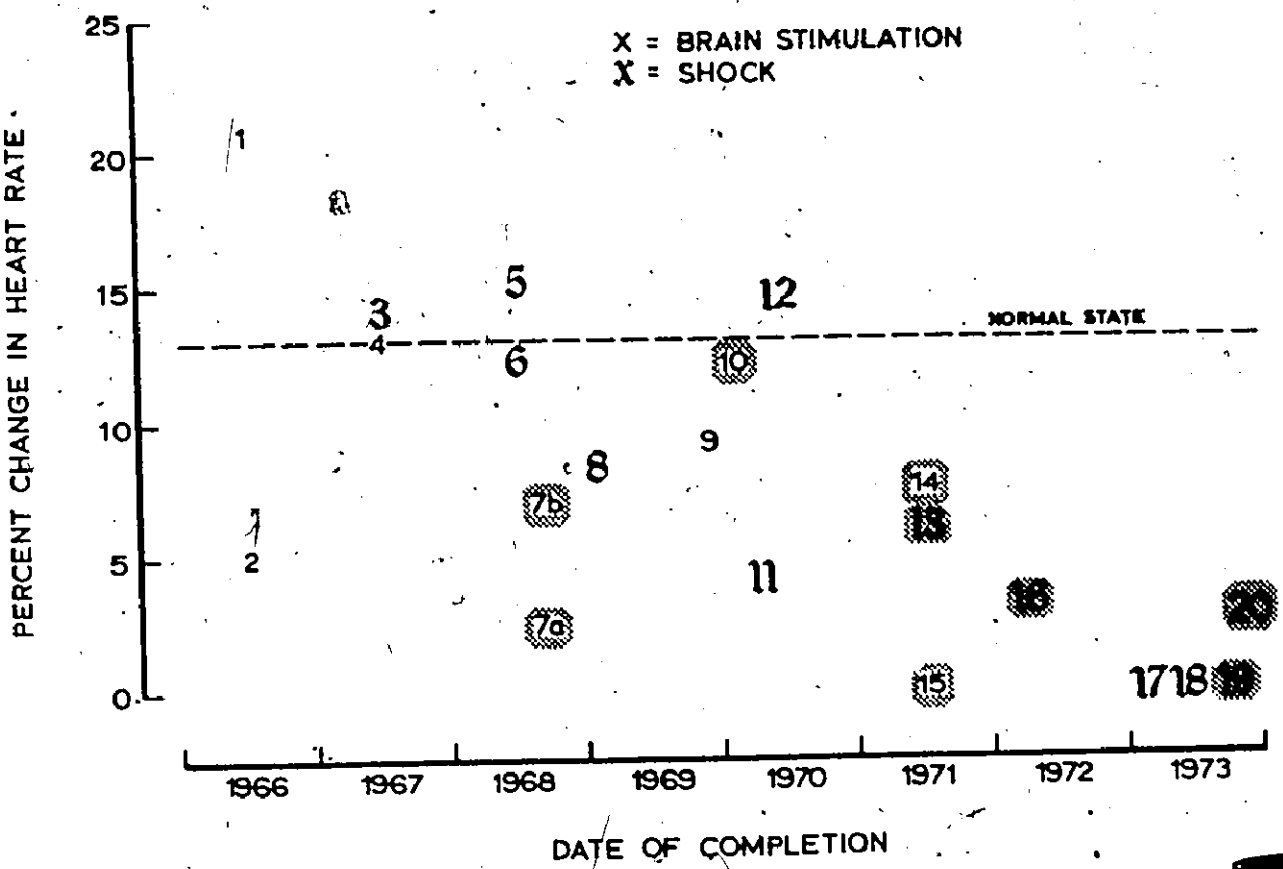
Hahn & Rinaldi, 1970; DiCara & Miller, 1968a) permitted the experimenter to manipulate punishment or reward criteria and are subject to the same criticism. Convincing evidence for operant conditioning of heart rate in the paralyzed rat is unlikely until the experimenter is removed from the experiment, and the shaping procedure is totally automated.

Operant Conditioning: Current Status

The studies reported in this thesis were begun at a time when the question of the replicability of the earlier Miller and DiCara experiments was first being raised. The findings reported here have some bearing upon this question.

The first compelling aspect of the work from the Miller and DiCara laboratory was that highly reliable and repeatable bidirectional changes were produced. The early experiments of Miller and DiCara (1967) and Fields (1970b) report heart-rate changes of 21% and 15% respectively by the end of punishment training. However, in the present experiments, the magnitude of the bidirectional difference, when a difference was produced, was only about 3%. This finding is supported by the results of other investigators as well. These are summarized in Figure 19 where heart-rate changes obtained in other laboratories are displayed as a function of the year in which they were reported (from 1966 to 1973). Inspection of this figure indicates that current investigators are not able to produce large changes in heart rate employing operant procedures in the paralyzed preparation. Furthermore, the tendency for the magnitude of the effect to decrease over time (Miller & Dworkin, 1974) is vastly diminished when only data from laboratories other than Miller and DiCara are considered.

FIGURE 19 - Magnitude of heart-rate changes in all published studies of operant heart rate conditioning in paralyzed rats.


X = BRAIN STIMULATION
X = SHOCK
PERCENT CHANGE IN HEART RATE
25
20
15
10
5
0
NORMAL STATE
1
2
3
4
5
6
7a
7b
8
9
10
11
12
14
15
16
17
18
20
1966
1967
1968
1969
1970
1971
1972
1973
DATE OF COMPLETION

The earlier experiments (DiCara & Miller, 1968a; Fields, 1970a,b) report that heart-rate changes in the rewarded direction were obtained in 100% of the subjects tested. This is certainly a marked contrast to the experiments of this thesis. Of the total of 114 rats were prepared, 25.4% died or were discarded because their heart rates never stabilized, and of the remaining subjects only 62.6% showed changes in the direction of training. Like the results summarized in Figure 19, the findings of the present experiments indicate that operant heart-rate conditioning in the paralyzed rat is not a highly reliable and repeatable phenomenon as was once believed.

Miller (1969) also asserted that larger heart-rate changes could be produced under curariform paralysis than in the normal state. This issue cannot be answered diractly by the studies in this thesis as none of the learning experiments was carried out in the normal state. However, DiCara & Miller (1969b) conducted on operant conditioning experiment in the nonparalyzed rat. The heart-rate changes produced in that study are indicated by the horizontal dotted line in Figure 19. Inspection of this figure clearly reveals that heart rate changes produced under curare are in fact smaller in most cases than changes produced in the normal state. Thus, there exists very little evidence in support of Miller's statement.

It should be noted that curare was first used by Miller and DiCara to rule out mediation of the heart-rate response by movement processes during operant conditioning. However, this interpretation can be challenged on two grounds, one interpretation and the other empirical. First, curare only blocks the neuromuscular junction. Goesling and Brener (1972) demonstrated that central movement processes still influenced heart rate even while the rat was paralyzed. For this reason, the curarized rat work

only rules out overt movement as a determinant of the heart-rate change. The basis of the second challenge concerns the instability of the original phenomenon. The failure to demonstrate operant conditioning in the paralyzed rat precludes any statement that such learning is possible in the absence of overt skeletal mediators.

Many investigators over the past six years have attempted to operantly condition large changes in the heart rates of paralyzed rats. All of these researchers acknowledge that the difficulties associated with the paralyzed rat preparation are numerous and not easily overcome (Obrist, Black & Brener, 1974). Furthermore, operant conditioning experiments that have employed this preparation have met with at best, only marginal success. After much effort, the phenomenon still remains undemonstrated. These reasons question the value of further operant conditioning experiments under curare and suggest that the issues that this line of research was developed to answer should be approached in another way.

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