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OPERANT HEART-RATE CONDITIONING IN THE CURARISED RAT

E. W. THORNTON

A thesis presented for the

Degree of Doctor of Philosophy

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A B S T R A C T

Many two-process learning theorists have attempted to separate the processes of operant and classical conditioning on the basis that the type of response each governed was typically different. Specifically, it has been postulated that responses of the autonomic nervous system are not subject to the direct influence of operant contingencies.

Within a bidirectional experimental design, it was shown that both increases and decreases in heart-rate could be produced by operant training procedures. The considerably easier task of shaping increases in rate was attributed to the interaction of the schedule and the unconditioned effects of the reinforcer on heart-rate. However, the results of the training procedures on the heart-rate were shown not to be a direct consequence of the presentations of the reinforcer.

In these studies curarised animals were used in order to rule out the possibility that the heart-rate changes were mediated by operant manipulations of overt and covert skeletal responses. Caution was stressed in the use of curariform drugs in studies of autonomic conditioning both because of their possible blocking action on autonomic ganglia and because of the difficulty in applying adequate and consistent artificial ventilation to the animal.

Experimental attempts were made to determine the specific mechanisms responsible for the operant heart-rate changes which were obtained. The evidence implied that increased output from both the sympathetic cardiac nerves and the adrenal medulla were

responsible for maintaining the increases in heart-rate. This evidence, together with the results of an experiment which showed depletion in levels of adrenal adrenaline as a consequence of the baseline procedures of curarisation and artificial ventilation, suggested that the procedures involved in these studies were stressful to the animal, producing increased sympathetic functioning.

The difficulty in resolving the problem of whether operant and classical conditioning are separate processes was not resolved by these experiments because of the possibility of central nervous system responses being influenced by each type of conditioning. The implications of these studies interpretation of autonomic response changes is considerable and the probable importance of operant components in studies of classical conditioning were elaborated.

C O N T E N T S

CHAPTER ONE

INTRODUCTION

In 1928, Miller and Konorski elaborated a point of view which is the basis of two-process learning theories. Their concept arose out of the idea that there are two basic forms of conditioning processes, Pavlovian or classical, and Thorndikian or operant. In Pavlovian conditioning, the experimenter arranges the relations between stimulus events which he controls but, in an operant conditioning situation, the experimenter arranges the relations between the organism's behaviour and future stimulus events. Despite arguments which pointed out difficulties in separating these procedures (see Hilgard and Marquis, 1961), two-process theorists maintained that the underlying learning principles governing each type of conditioning could not be resolved. One of the major defences for this position was that the type of response each governed was typically different. Miller and Konorski distinguished between responses yielding little or no sensory feedback and those yielding rich sensory feedback. They assumed that Pavlovian conditioned reflexes yielded poor sensory and proprioceptive feedback but, in contrast, Thorndikian response learning involved extensive and intricate feedback mechanisms. Skinner (1938) stated, "glands and smooth muscles do not normally produce the kinds of consequences involved in operant reinforcement and when we arrange such consequences experimentally, operant conditioning does not appear."

The first really explicit statement of a two-process theory was formulated by Schlosberg (1937) who claimed that the empirical laws of Pavlovian conditioning were the laws of conditioning of diffuse responses of an emotional type, while Thorndikian learning implied that the major associative process was that linking the stimulus and the precise adaptive motor responses.

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Mowrer (1947) developed this basic premise in a more sophisticated manner in attempting to overcome the difficulties which arose in explaining the acquisition and maintenance of avoidance behaviour. An animal escaping from a punishing stimulus could be explained by drive reduction following removal of that stimulus. However, during avoidance learning, a successful response could not logically be reinforced in this manner by the absence of the aversive stimulation. Mowrer postulated the concept of Pavlovian conditioned responses acting as motivational mediators for the instrumental avoidance responding. He also maintained that Pavlovian conditioning applied only to visceral responses whilst Thorndike's law of effect applied only to the motor responses of skeletal muscles. The essential point of Mowrer's idea was that visceral responses created emotional or motivational tensions which were resolved by operant behaviour. These visceral responses, or conditioned emotional responses, produced afferent feedback stimulation which was suggested to have drive properties. The operant response was reinforced by a reduction in these visceral responses. The theory postulates intimate links between 'fear' states, the conditioned emotional responses and the autonomic nervous system. Presumably this was partially a consequence of the common observation that autonomically innervated effectors are seen to be active during periods of 'emotional' behaviour.

Full discussions of two-process theories can be found in reviews by Rescorla and Solomon (1967) and Herrnstein (1969), but the main points of importance for this thesis have been outlined above. Although other forms of two-process learning theories have been developed either in terms of the nature of the Pavlovian mediating responses (Solomon and Wynne, 1954) or, in the relationship between

reinforcing events and the learning processes (Spence, 1956; Mowrer, 1960), it is the Mowrer (1947) version which has generated the greatest proportion of experimental effort, in particular by suggesting two lines of research relevant to autonomic conditioning. To understand the importance of the line of experimentation presented in this thesis, it is necessary to elaborate both types of experiment.

1. Pavlovian Autonomic Mediation of Operant Behaviour

Many experimenters have made a direct attempt at elucidating the autonomic classical conditioned mediators of operant behaviour and determining what laws are governing their control. The ideal was to find some peripheral response index of emotional arousal or the conditioned emotional response. Dunbar (1954) has compiled an exhaustive list of early experiments which investigated the possible different response indices. The most frequently used index of emotional arousal has been heart-rate. The following more recent examples indicate that intensity of affect has been inferred from the magnitude of heart-rate changes (Lacey, 1956; Lykken, 1962; Church, Lolordo, Overmier, Solomon and Turner, 1966). Ax (1953) and Funkenstein, King and Diolette (1954) have made attempts to differentiate affective states, such as anger and fear, from heart-rate changes, and the direction of change of affect has also been inferred from heart-rate change (Black, Carlson and Solomon, 1962).

There are two basic assumptions behind such experiments:

(i) That there is a direct relationship between emotional arousal and the processes controlling peripheral responses of the autonomic nervous system.

(ii) That heart-rate changes directly reflect changes in these controlling processes.

These assumptions arose mainly from extensions of Cannon's classic experiments (Cannon and de la Paz, 1911; Cannon, 1914; Cannon and

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It is not intended to dispute that the heart-rate is changed by activity in sympathetic and parasympathetic nerves to the heart, but rather, that any change in central autonomic processes is not necessarily reflected in all the autonomic nerves at any one time. Changes in central autonomic processes should not be assumed to discharge as a single unit but rather ~~that~~ the activity of the autonomic nervous system may be seen as being more discrete, effecting changes in different peripheral autonomic nerves as the occasion demands.

Britton, 1927; Cannon, 1929) which indicated that the sympathetic nervous system (SNS) discharges as an integrated unit when bodily resources are suddenly mobilised for vigorous activity. The effect of this direct neural influence on the smooth and cardiac muscle within the body is supplemented by adrenaline, secreted from the adrenal medulla into the blood stream under sympathetic control. The parasympathetic nervous system (PNS) was suggested to have an antagonistic effect, tending to conserve body resources by its ability to influence single organs. The illogical argument followed that, since the heart is innervated by both the SNS and the PNS, its rate will reflect the balance between these parts of the autonomic nervous system and therefore will reflect the more central processes which control SNS and PNS activity.[1] Assumption (i) is embarrassed by a series of experiments which demonstrate that, although autonomic indicators are responsive to heightened emotional states, the correlations obtained between autonomic channels of reactivity are low (Wenger, 1942, 1948; Malmo and Davis, 1956; Clemens, 1957; Lacey and Lacey, 1958a, 1958b). Lazarus, Speisman and Mordkoff (1963) used a more sophisticated technique for the analysis of similar data in order to increase the correlations between autonomic responses, but despite this, concluded that the maximum correlation between various indices of autonomic nervous system was only moderate even under the most favourable conditions. However, Lacey, Bateman and Van Lehn (1953) and Engel (1960) have postulated specific sources of this variance in autonomic activity and maintain the idea that the autonomic nervous system is always active in emotional arousal. They criticise the data showing low correlations in autonomic response indices obtained in the studies quoted, because the data was gathered from different subjects in diverse situations. They have shown, with moderate success, larger correlations if measures

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are taken from the same subject in the same situation on different occasions.

Assumption (ii) requires that heart-rate changes reflect processes controlling autonomic activity, yet is not always substantiated by experimental evidence. Rushmer (1961) and Robinson, Epstein, Beiser and Braunwald (1966) present data which demonstrates that identical changes in heart-rate can reflect very different patterns of autonomic activity. Moreover, in accordance with Wilder's (1956) Law of Initial Values, differential activation of either portion of the autonomic nervous system can produce different directions of change in heart-rate in a manner determined by the resting baseline level. This law states that the higher the resting baseline heart-rate, the lower the increase in rate which is produced by a given increase in sympathetic activity. Paradoxically, if the baseline level is very high, an increase in sympathetic activity to the heart may cause a decrease in heart-rate.

Despite lack of experimental justification for the basic assumptions, heart-rate has been used many times in attempting to reveal some of the properties of Pavlovian mediators of operant behaviour. Rescorla and Solomon (1967) have reviewed many of these experiments. Not surprisingly, the agreement in the results is low and Rescorla and Solomon rightly conclude, "that the relationship between overt operant behaviour and heart-rate change (if any really exists) is easily disturbed by unidentified variables". The physiological mechanisms for control in the cardiovascular system and the factors affecting cardiac rate have been well documented (see Rushmer, 1958; Rushmer and Smith, 1959; Best and Taylor, 1966). It can be concluded, as a consequence of this literature, that the relationship between gross behaviour and changes in the cardiovascular system is likely to be most complex, since many self-regulatory mechanisms are involved in that system.

2. Operant Conditioning of Autonomic Responses

Mowrer's (1947) specific formulation of a two-process theory requires, a priori, that responses controlled by the autonomic nervous system be subject only to classical conditioning and that skeletal responses should not be directly modified by similar laws. However, it is not a logical necessity that these response classes and reinforcement should be separated in an absolute and exclusive manner. If both the above possibilities occur for any response, then we cannot rely on the interaction of reinforcement contingencies with the distinction between autonomic and skeletal responses to justify the theoretical separation of the effects of stimulus and response contingent reinforcement.

This thesis is primarily concerned with the investigation of one of these possibilities, that is, whether it is feasible to demonstrate operant conditioning of an autonomic response. Success would question two-process theory in the form postulated by Mowrer (1947). It would also suggest even less justification for the studies which attempt to investigate the autonomic mediators of instrumental behaviour, since these assume autonomic responses are not under the control of operant principles. A positive study, showing operant conditioning of an autonomic response, would not destroy two-process learning theories, it would only affect those which specifically state that the mediation of instrumental responses is through classically conditioned, autonomic responses.

Despite the specificity of the problem, virtually no early experimental work was undertaken. Skinner (1938) is the only early reference and his experiment was described in two sentences:

"I have attempted to condition vasoconstriction of the arm in human subjects by making a positive reinforcement depend upon constriction. The experiments have so far yielded no conclusive result".

More recently a great deal of experimental effort has been aimed at resolving the problem, Most of this work has been performed with human subjects using a diversity of response indices of autonomic activity. These studies have been reviewed by Kimmel (1967) and Katkin and Murray (1968). They are confounded by an acute problem that is raised in a direct attempt to validate the hypothesis. This concerns the question of whether any changes produced in the appropriate response are a direct consequence of the operant procedure. There is the possibility that such changes may be mediated by operant conditioning of some other skeletal response, which, in turn, produces a change in activity of the autonomic response being studied. This controversial point was highlighted by Smith (1954) who claimed that the autonomic nervous system is solely an efferent system and lacks any afferent function and is, therefore, incapable of learning by operant reinforcement principles, since dependable response associated cues are necessary conditions for the establishment of operant learning. This point is not, however, always justified, since there is considerable evidence for afferent feedback to the central nervous system for changes in response activity within the cardiovascular system. The best known of these is afferent feedback of blood pressure changes through the activity of the baroreceptors in the carotid sinus and the walls of the aortic arch. Rushmer (1961) in an authoritative article, suggests that it is probable that there are other mechanisms for afferent feedback of changes within the cardiovascular system. The acute point of Smith's argument is that skeletal activity will influence autonomic responses, especially those of the cardiovascular system. Such effects have led to confusion in the literature. For example, Razran (1961) reported evidence that Lisina, a Russian worker, had conditioned vasodilation instrumentally. However, Lisina (1965) did not support this claim

rather she concluded that her subjects were able to gain voluntary control over their blood vessels by using a number of special learnt responses. The most frequent of these responses was changing the depth of respiration and the relaxation of the skeletal musculature. Murray and Katkin (1968) analysed the verbal reports of the voluntary actions of subjects who had participated in two studies of operant conditioning of heart-rate changes performed by Engel and Hansen (1966) and Engel and Chism (1967). The analysis showed a high correlation between specific reports and the heart-rate changes which had been obtained. Moreover, these high correlations were obtained irrespective of whether the reinforcement contingencies had been used to condition either increases or decreases in rate. There have been several suggestions of possible ways in which changes of autonomic responses, apparently under direct operant control, may be causally or concurrently associated with skeletal conditioning and/or previous classical conditioning. These possibilities have been elaborated in some detail by Smith (1954, 1964); Black (1967) and Katkin and Murray (1968).

To avoid the problems associated with the skeletal mediation of autonomic responses, this thesis has concentrated on experiments which have used animals paralysed by a curariform drug in order to produce a flaccid musculature. These effects of the drug consequently require that the animals be artificially ventilated. This prevents the unstable or voluntary respiration changes influencing the autonomic response, a possibility which is an acute danger, since Westcott and Huttenlocher (1961) and Wood and Obrist (1964) have demonstrated that changes in the depth of respiration produced considerable effects on the variability of human heart-rates. To completely rule out the possibility of skeletal mediation of the

autonomic response, the dose level of the curariform drug must be sufficient to suppress not only overt activity but also covert activity of the muscles. This is required in the light of a demonstration by Black (1966) that, in dogs, learning of skeletal responses which showed up in the EMG but not as gross overt movements, could influence heart-rate responses. A similar point has been suggested in an earlier article by Smith (1964).

The emphasis of the thesis is on cardiovascular responses, in particular, heart-rate. This is because the evidence for an afferent function for such responses, a prerequisite for operant conditioning, is most convincing. The choice also has a practical advantage in that a response such as heart-rate is easy to record and is readily quantifiable.

CHAPTER TWO

OPERANT CONDITIONING OF HEART-RATE CHANGES IN THE CURARISED ANIMAL - 1.

This chapter presents a study of operant conditioning of heart-rate changes in the curarised rat. It was undertaken for reasons which have been elaborated in the introduction.

The aim of the experiment was to determine if it were possible to train the heart-rate response of the deeply curarised rat to escape and avoid a mild electric shock. This particular schedule was chosen from both practical and theoretical considerations. The problem of choosing a reinforcer suitable for use with the curarised rat is considerable. A consistently effective reinforcing stimulus is required which does not need any skeletal motor behaviour on the part of the organism in order to operate. For theoretical reasons, the stimulus must also have its effect as immediately as possible. Two alternatives seem suitable, electric shock, and intracranial stimulation in either positive or negative reward areas of the brain (see Olds, 1958). Electric shock was chosen because it has the advantage that it does not require operant lever press pretraining, in the non-curarised state, to test its effectiveness prior to its use in the operant conditioning of heart-rate changes.

An experimental design was used in which half the animals were to be trained using operant principles, such that heart-rate increases above a criterion level in the presence of an operant conditioning stimulus (CS+), would avoid the shock. This group was to be called the increase group. The other half of the animals were trained, using similar principles, such that heart-rate decreases below a criterion level, in the presence of a similar operant stimulus (CS+) would avoid the shock. This group was to be called the decrease group. In this bidirectional design, the two groups were expected to display a

difference in response level at the end of training if shock presentation and avoidance of shock were dependent on response rate. If, however, no such relationship of response levels were to occur, then any changes in the heart-rate responses which did occur would be caused by variables other than the dependency between response and reinforcement. These changes could be produced by either the presentations of the shock, or by pairing of the shock and the operant stimulus. If the number of such presentations and pairings were the same in the increase and the decrease groups, then the two groups should display similar response rates.

Incorporated into the experimental design was the operant principle of shaping by successive approximations to the desired response. Each animal, at the start of training, was to be set a criterion level for avoiding or escaping the shock which was within the normal variation of the heart-rate response of that animal under the conditions of curarisation and artificial ventilation. When, during training, the animal was successfully avoiding this criterion level, it was shifted to a criterion level which was slightly more difficult. These shifts were to be continued throughout training. However, if the shift at any one time was to prove too difficult, then the criterion level would be returned to the level which the animal had previously attained.

EXPERIMENT

METHOD

Subjects

Sixteen rats (ten female, six male) weighing between 200-250 gm. were taken from the colony maintained in the Department of Psychology, University of Durham. Four of these animals (three female) were discarded as they failed to attain a stable baseline heart-rate response during the adaptation period.

Apparatus

(i) Enclosure for operant training procedure

During operant heart-rate training the curarised rat lay on its side on a metal plate, in which a hole had been cut to allow faeces and urine to fall into a space below (D. Figure 2.1). The plate was supported two centimetres above the floor of the experimental enclosure by four rubber bungs and the rat was insulated from the plate by a thin plastic pad.

The experimental enclosure was a sound-proof, black box 40cm. wide 45cm. high and 32cm. deep. The enclosure was fitted with a 60 watt lamp (A. Figure 2.1), situated 15cm. above the animal and a loud speaker (C. Figure 2.1) 10cm. from the subject, at an angle of 30°, was set to deliver white noise of 78 decibels intensity (ref. 0.002 dyne/cm^2).

Shock was applied through two wires, wrapped around the tail 2cm. apart, approximately two-thirds from the base of the tail. The shock generator was set to deliver pulses of a fixed length of 0.1 seconds and a fixed intensity of 0.5mA.

Electrodes for recording heart-rate were stainless steel needles, situated subdermally on either side of the thorax. The indifferent electrode was a hypodermic needle inserted into the intraperitoneal cavity and connected, via polythylene tubing, to the outside of the chamber. This arrangement allowed additional doses of d-tubocurarine chloride to be given to the animals during operant training without disturbing them.

During the experiment, artificial ventilation was maintained via a snout mask, which was made from the mouthpiece and neck, cut from a child's balloon. The lower lip of the mouthpiece was placed behind the upper inscissors of the rat and the upper lip fitted snugly over the top of the snout. The end of the neck of the balloon distal from the mouthpiece, was stretched over a rubber bung. The mask was

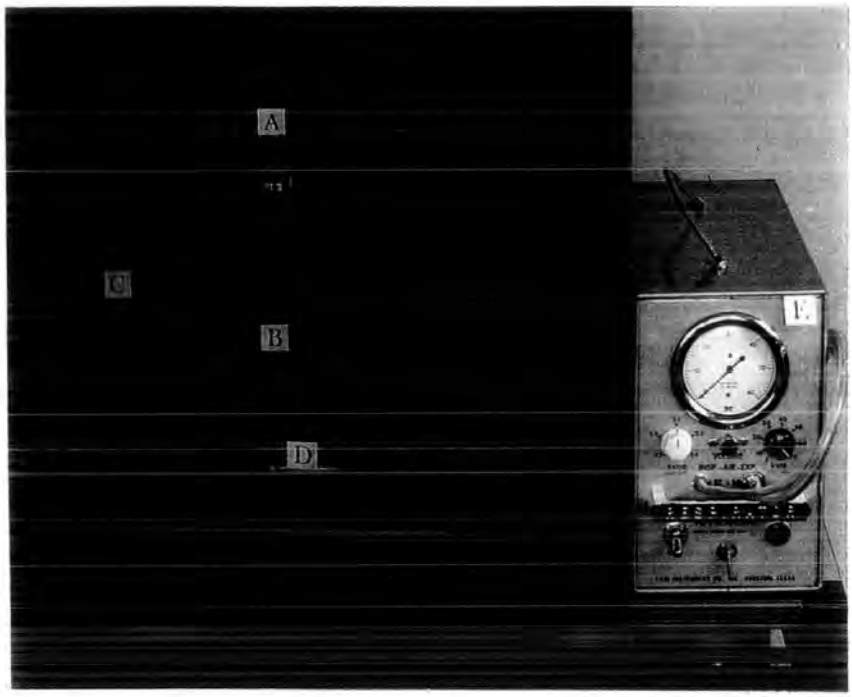

FIGURE 2.1 The enclosure for operant training procedures in curarised rat.

A = A 60 watt lamp used as one conditioning stimulus.

B = Mouthpiece into which the snout of the rat was fitted for artificial ventilation.

C = A loudspeaker from which white noise of 78db acted as a second conditioning stimulus.

D = Hole to allow faeces and urine to fall away from the rat.

E = A small animal respirator (the respirator shown (E. and M. Instrument Co.) was not used in the present experiment but was later chosen in preference to the respirator with passive expiration and was used in all subsequent experiments presented in this thesis).

connected to the inlet and outlet tubes of a small animal respirator, with passive expiration, by a 'Y' piece of glass tubing, the stem of which was pushed through the centre of the bung. To faciliate this procedure, the bung had been previously drilled through the centre. The arrangement for artificial ventilation of the rat is presented in detail in Figure 2.2. Figure 2.3 shows the rat in the enclosure during experimental conditions when the snout had been fitted into the mask and recording and shock electrodes attached to the animal.

(ii) Reinforcement circuit for determining criterion heart-rate levels.

The electrocardiograph (EKG) was monitored with an Alvar Electroencephalograph. An output was taken, via a jackplug, from the terminal stage of the power amplifier in the electroencephalograph and passed through a sensitive electronic pulse former whose final stage operated a reed relay. The amplifier in the electroencephalograph was adjusted, so that the reed relay was only operated by the QRS wave of the EKG signal. The pulse length of the electronic pulse former was adjusted to give a square wave pulse of sufficient length to activate a conventional electro-mechanical pulse former, and the formed signal from this last stage was then used to operate the criterion and counter circuitry which were of the conventional electro-mechanical type. For four animals, a simultaneous direct cardiotachometer reading was obtained on a Beckman type RP Dynograph.

The circuitry for determining whether the heart-rate of the animal was above or below a certain criterion rate consisted essentially of two predetermining counters (Sodeco) and appropriate pulse formers with a changeover switch circuit. One of these counters was driven by a ten pulse-per-second generator and acted as an accurate timer. This timer could consequently be varied by fractions of one-tenth of a second. The second counter was activated by the formed EKG signal derived from the animal.

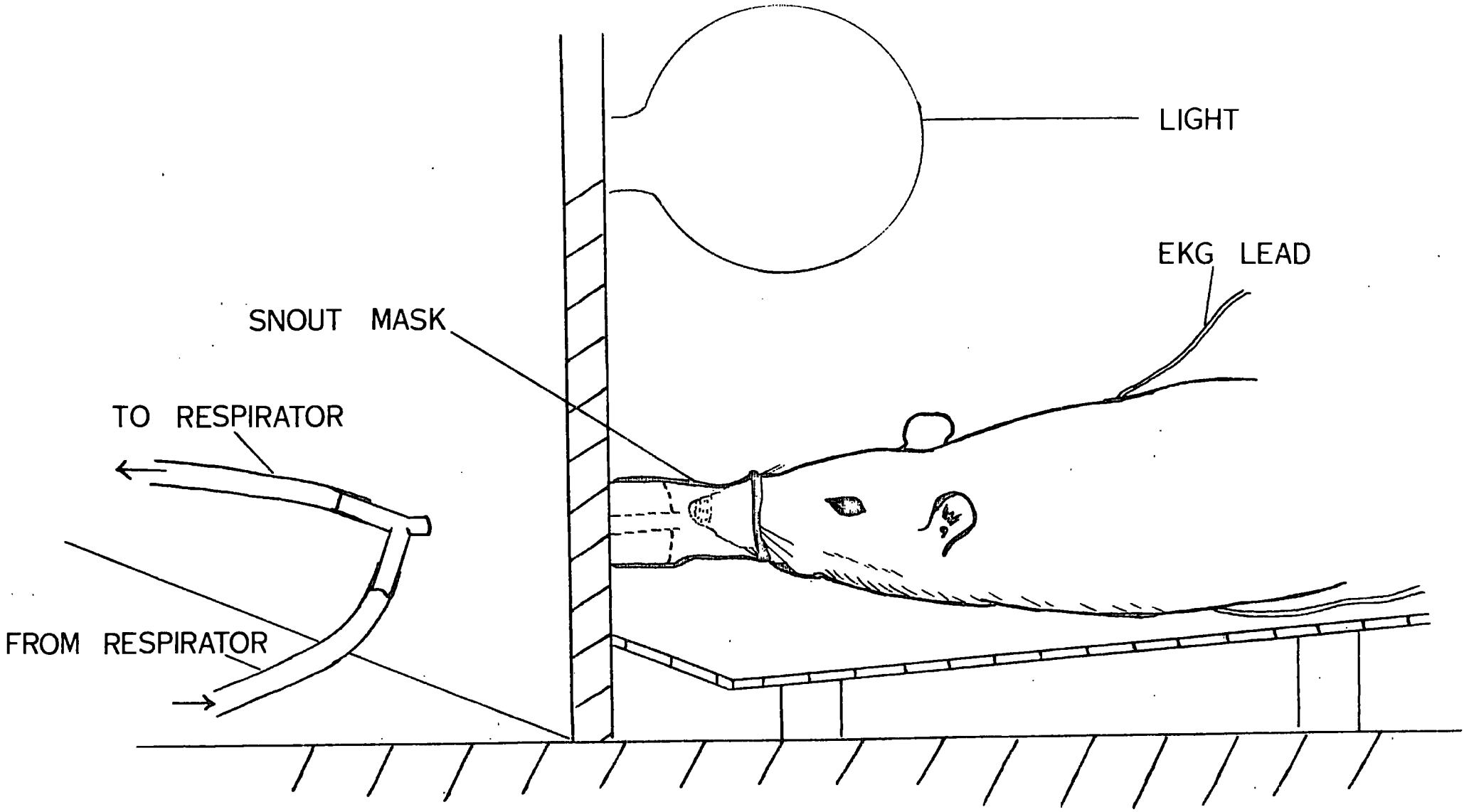

FIGURE 2.2 A detailed diagram of the apparatus for artificial ventilation of the curarised rat

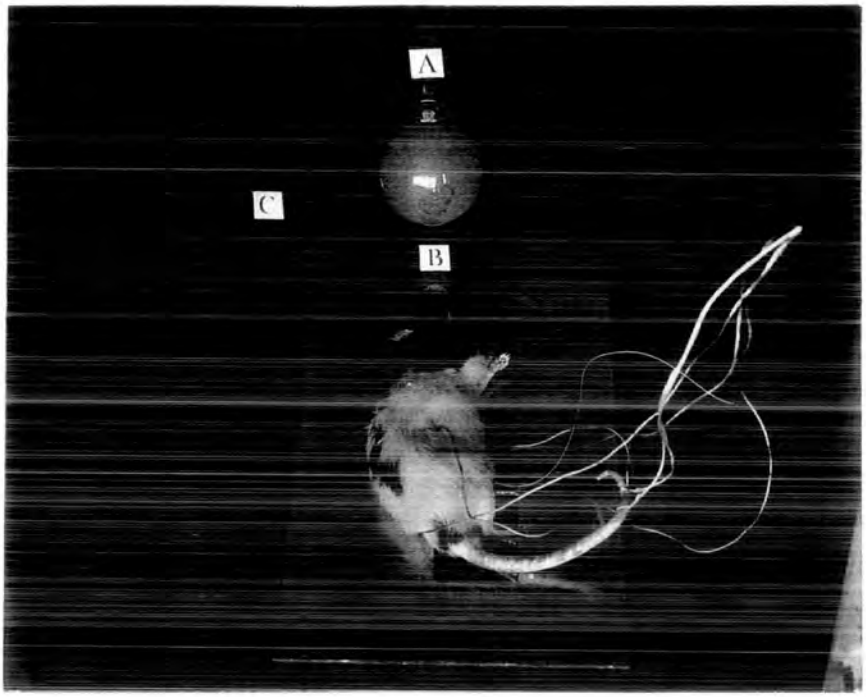

FIGURE 2.3 The curarised rat fitted into the snout mask in the operant training enclosure. The electrodes for recording the EKG and for delivering shock to the animal's tail can be seen attached to the animal.

On an operant conditioning trial, when the appropriate signal (CS+) came on both counters were activated (through a logic AND-gate relay) at exactly the same time. By adjustment of the counter settings, in a manner determined by a simple table (Table 2.A), it could be determined whether the heart-rate, sampled over several inter-beat-intervals (IBI), or part thereof, was faster or slower than the fixed rate of the timer. The length of time of these samples ranged between 1.7 and 2.3 seconds as determined by setting the counter which acted as the accurate timer. This variation was necessary in order to be able to increase or decrease the criterion level by approximately two percent intervals. On practical grounds, it was necessary to program the criterion circuit so that a single output occurred if the two counters reached zero at the same time. This output was chosen to indicate that the criterion level had not been reached.

The apparatus was programmed such that if the criterion level was reached in one cycle of the predetermining counters, then the signal for an operant training trial was terminated and the criterion circuit was inactivated until the next operant trial. However, if the criterion level was not reached, the two predetermining counters were reset when the inappropriate timer reached zero. The cycle was then re-started by the formed EKG signal and the fixed timer through the AND-gate relay. If the criterion level had not been attained within a period of five seconds, then an 0.1 pulse of shock was programmed to be delivered at the termination of the counter cycle in operation at the end of that period, provided criterion level was not attained. This shock continued to be administered at the end of each consecutive cycle of the counters in an 0.1 second pulse until the criterion level was attained. The signal for the operant training trial was then terminated. As a consequence of this procedure, the actual length of each operant trial was to be determined by an

TABLE 2.A Table to determine the counter settings for assessing criterion level heart-rates. Counter 1 is driven by the heart-rate of the animal. Counter 2 is driven by a fixed ten pulse per second generator.

Counter 1 Setting	Counter 2 Setting	Heart-Rate in Beats Per Minute	Counter 1 Setting	Counter 2 Setting	Heart-Rate in Beats Per Minute
17	20	510	15	23	319.5
16	19	505	13	20	390
15	18	500	11	17	388
19	23	495.5	14	22	382
14	17	494	12	17	379
18	22	491	13	21	371.5
17	21	485.5	11	18	367
16	20	480	14	23	365
15	19	473.5	12	20	360
18	23	469.5	13	22	354.5
14	18	466.5	10	17	353
17	22	463.5	11	19	347.4
13	17	459.0	12	21	343
16	21	457.5	13	23	339
15	20	450	10	18	333
17	23	443.5	11	20	330
14	19	444.2	12	22	327
16	22	436.5	9	17	317.5
13	18	433.5	10	19	316
15	21	428.5	12	23	313
12	17	423.5	10	20	300
14	20	420	11	23	287
16	23	417	10	21	285.5
13	19	410.5	9	19	284
15	22	409	8	17	282.5
14	21	400	10	22	272.5

interaction of both the programmed circuitry and the actual heart-rate of each animal at that time. Figure 2.4 shows some examples of this interaction and its effect on the length of an operant trial. Throughout training, every tenth operant trial was a test trial in which the operant trial stimulus came on for exactly five seconds during which the criterion circuit was inactivated. The number of heart beats occurring within this period was recorded by a print-out counter.

Two other types of trial, a non-reinforced trial (CS-), in which a second stimulus was presented for five seconds, and a blank trial during which no stimulus occurred, were also programmed into the operant training schedule. In both these types of trial, heart-rate was recorded in print-out form. It was hoped that incorporation into the training schedule of such trials would provide evidence of discrimination in the heart-rate response of animals between reinforced and blank trials.

Operant training of heart-rate changes was programmed to consist of two hundred and forty trials, divided equally into each of the three types of trial. Trials were presented with an inter-trial-interval of 20 seconds, and the same cycle pattern of trials was repeated consecutively every thirty trials. Within each block of these thirty trials, each trial occurred in a predetermined random schedule with the following constraints:

(a) There were no more than two consecutive trials of any one type.

(b) Each type of trial was not separated from a similar trial by more than four other trials.

Procedure

The rat was injected intraperitoneally with 0.6 mg/Kgm body weight of d-tubocurarine chloride. This dose level, when supplemented

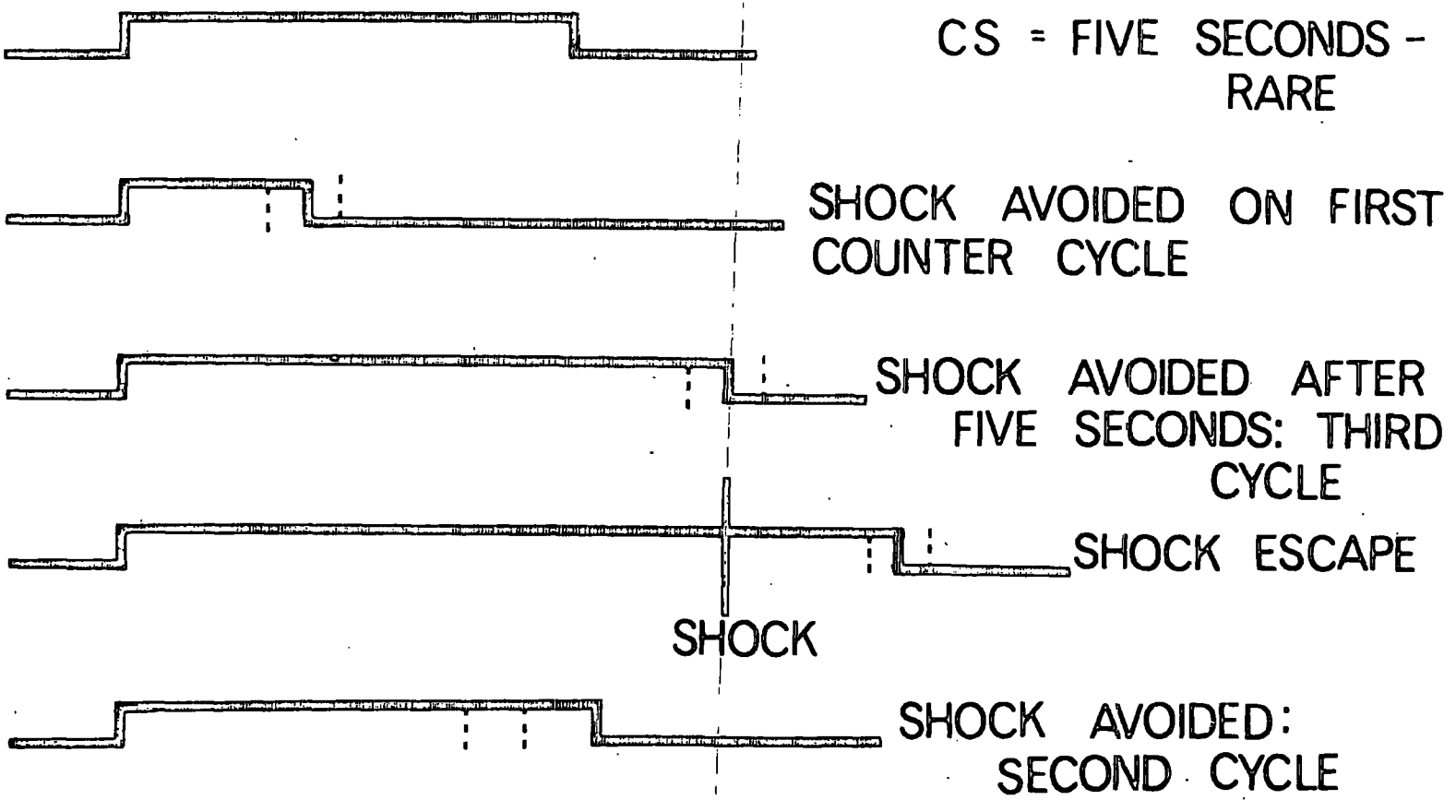

FIGURE 2.4 Shows some examples of the length of time during which the CS+ was presented to the curarised animal during any one operant training trial. The length was determined by an interaction of the programmed interval of five seconds for the CS+ and the attainment of criterion level, as determined by the relative speeds of the predetermining counters, the variability of which was determined by the rat's own heart-rate. When the criterion level was attained within five seconds, shock was avoided and the CS+ terminated. When the full five seconds had elapsed and criterion level had not been reached, the consequence depended on the counter cycle in operation at that time. If, on completion, criterion level was attained, then CS+ terminated and shock was avoided; however, if criterion level was not attained, a shock pulse was delivered and the counter cycle re-activated. Escape from shocks occurred when criterion level was attained. The dotted lines in the figure indicate variations in the activity of the cycle of counters since they were only activated when relevant pulses occurred at exactly the same time.

with additional doses of 0.06 mg/Kgm of d-tubocurarine chloride injected through the indifferent electrode every six minutes throughout the experimental period, had been shown by preliminary studies to completely suppress EMG activity recorded from the gastrocnemius muscle. When the rat became flaccid and showed signs of respiratory failure, it was fitted into the snout mask and ventilated at seventy respiratory cycles per minute, with a peak pressure of fourteen centimetres of water (the procedure of artificial ventilation is described fully in Chapter Four). The temperature of the chamber was maintained at approximately 30°C to prevent loss of body temperature due to inactivity of the animal. The electrodes for recording heart-rate were inserted subdermally under topical applications of xylocaine anaesthesia. The shock electrodes were attached to the tail and the experimental chamber closed. The rat was then allowed a period of thirty minutes for the animal's heart-rate to stabilize.

Animals were initially allocated to either the increase or decrease group on a random basis but, after the first four, an attempt was made to balance the two groups with rats having similar baseline heart-rates. The rats were further assigned to a subgroup in which either the light or the tone was the signal for the operant, CS+ trial. The non-reinforced, CS- trial stimulus was the alternative stimulus. For three rats in each group, the light served as the CS+ stimulus and the tone as the CS- stimulus.

After the thirty minutes stabilization period, the programmed schedule was activated. Initial criterion levels were selected so that they would be achieved approximately four out of ten cycles of the counters. If the animal successfully avoided shock for four consecutive CS+ trials, the criterion level was made more difficult (by approximately 2 to 3 percent). If more than thirty 0.1 second pulses of shock were received in two consecutive CS+ trials, then the

criterion level was made easier by an equivalent amount of 2 to 3 percent.

When the training schedule had been completed, the programming apparatus was turned off and the additional injections of d-tubocurarine were terminated. Recovery of the animal from paralysis occurred after periodsof time ranging from 45 to 110 minutes. After the animal had struggled free of the snout mask it was carefully observed for ten minutes before it was returned to its home cage.

RESULTS

The 240 training trials were analysed in eight blocks of thirty trials. It should be remembered that each block of training trials consisted of the same distribution of ten CS+, ten CS- and ten blank trials. The heart-rate, during CS+ trials, was ascertained from only the last of those trials. Means were computed for the heart-rate obtained during CS- and blank trials for each animal in each block of trials. Subsequently, calculations were made of the mean heart-rate changes of the six animals shaped to produce heart-rate increases, in the CS+ (test), CS- and blank trials, in each of the eight blocks of training trials. The change in these mean heart-rate responses over the successive blocks of training trials, is presented graphically in Figure 2.5.

The group mean increase in heart-rate was from 396 beats per minute to 449 beats per minute. Similar calculations of mean heart-rate responses were made for the six animals shaped for heart-rate decreases, and the change in these values over successive blocks of training trials is shown in Figure 2.6. The group mean heart-rate response for the decrease group was 414 beats per minute at the beginning and the end of training.

The percentage change in heart-rate over the training period during CS+ trials, for each animal, is presented in Table 2.B. As

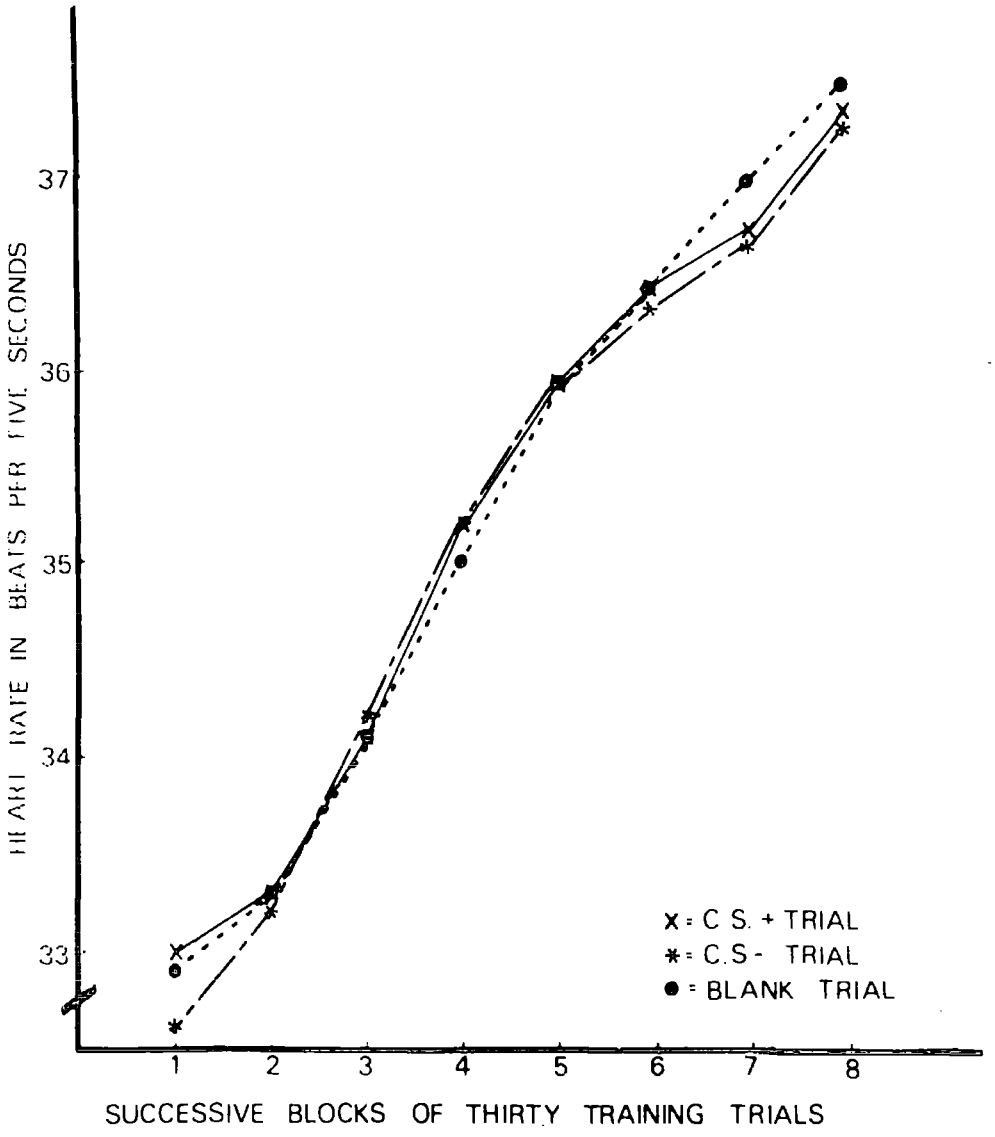

FIGURE 2.5 The mean changes in heart-rate of curarised rats shaped for heart-rate increases during reinforced, non-reinforced and blank trials, over the eight successive blocks of training trials. There is an increase in rate during each trial throughout the training trials.

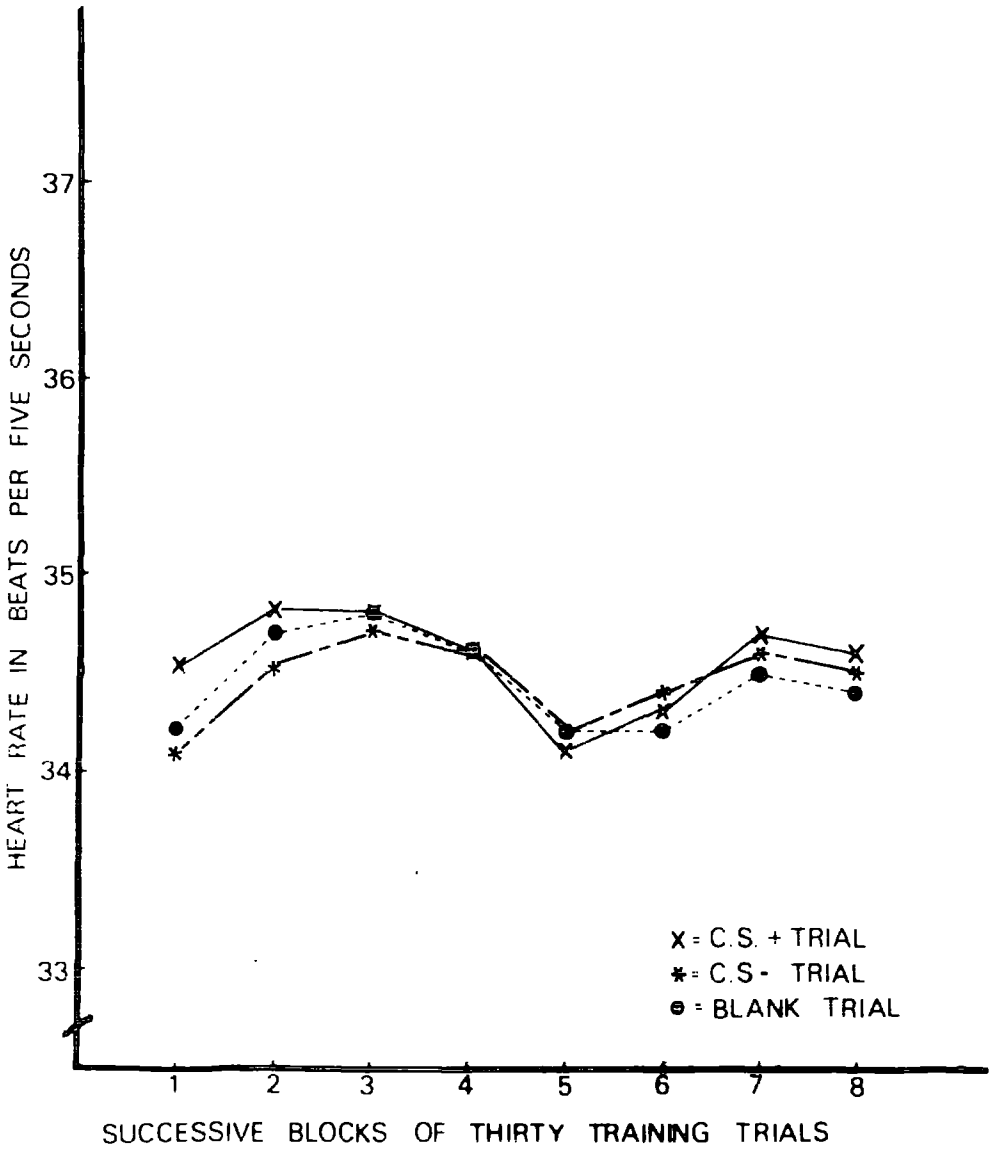

FIGURE 2.6 The mean changes in heart-rate of curarised rats shaped for heart-rate decreases during reinforced, non-reinforced and blank trials, over the eight blocks of training trials. There is apparently little change in rate during any trial over the training period.

TABLE 2.B The percentage change in heart-rate over training attained by each animal, together with the frequency and probability of receiving shocks during training by that animal.

Column headings:

A = Percentage change in heart-rate over training trials. Negative numbers indicate a decrease in rate.

B = The mean number of shocks received per block of ten operant trials throughout training.

C = The mean number of trials per block of ten operant trials on which a shock was received during training.

L = Light serves as operant, CS+ stimulus.

N = White noise serves as operant, CS+ stimulus.

increase group				decrease group			
subject	A	B	C	subject	A	B	C
1 L	33	10·4	3·7	1 L	0	51	3·5
2 N	16	20·4	4·5	2 N	8	36	3·7
3 L	10·3	16·4	5·6	3 L	-6	37	3·1
4 N	13·2	22	5	4 N	-3	56	3·7
5 L	16	15	4	5 L	0	18·5	2·5
6 N	0	36·4	4·7	6 N	0·2	29	3·6

can be seen, there appears little consequence as to which of the stimuli served as the stimulus for the operant, CS+ trial. A t-test on the difference in percentage changes in heart-rate produced in animals in the decrease group compared to the animals in the increase group, gave a value of $t = 3.11$, df 10; $p > 0.02$, < 0.01.

The effect of the operant training schedule can be seen, from this data, to have produced an apparent increase in heart-rate response for animals in the increase group, but little effect on animals in the decrease group. Reference to Figures 2.5 and 2.6 would indicate no apparent differences in the heart-rate changes produced between CS+, CS- and blank trials within either the increase or decrease groups. This was confirmed by a trend analysis.

There was an apparent difference between the increase and decrease groups in the mean number of shocks received per block of ten operant training trials by each animal. This parameter, one of shock frequency, is presented for each animal in Table 2.8. A test of the apparent difference in this parameter between the two groups gave a value of $t = 2.64$, 10 df; $p > 0.05$, < 0.02. The difference is most clearly seen in Figure 2.7 which presents a plot of the mean number of shocks received per block of training trials, and the percentage change in heart-rate over the training period, for each animal. The plot shows that the increase group animals tended to receive fewer shocks per block of training trials. Figure 2.8 presents a graph demonstrating the change in the group means of the number of shocks received per block of trials for each of the two groups, over successive blocks of training trials. Although the shock frequency for the decrease group is much higher in the first few blocks of trials, the frequency is reduced in later blocks. In contrast, the shock frequency for the increase group tends to be more stable over the blocks of trials.

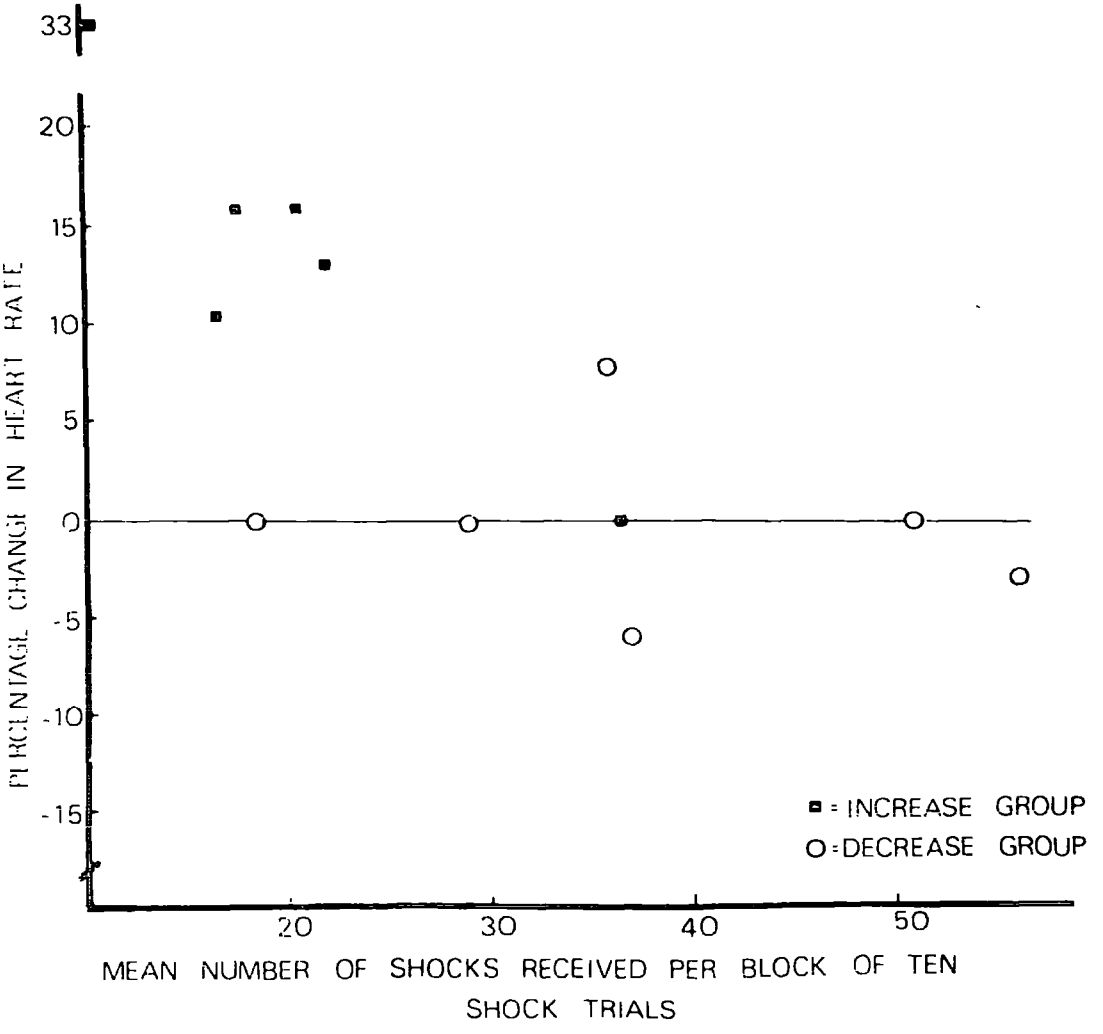

FIGURE 2.7 A plot of the mean number of shocks received per block of ten operant training trials, and the percentage change in heart-rate over the training period, for each animal. The plot indicates that fewer shocks were received by rats shaped for heart-rate increases.

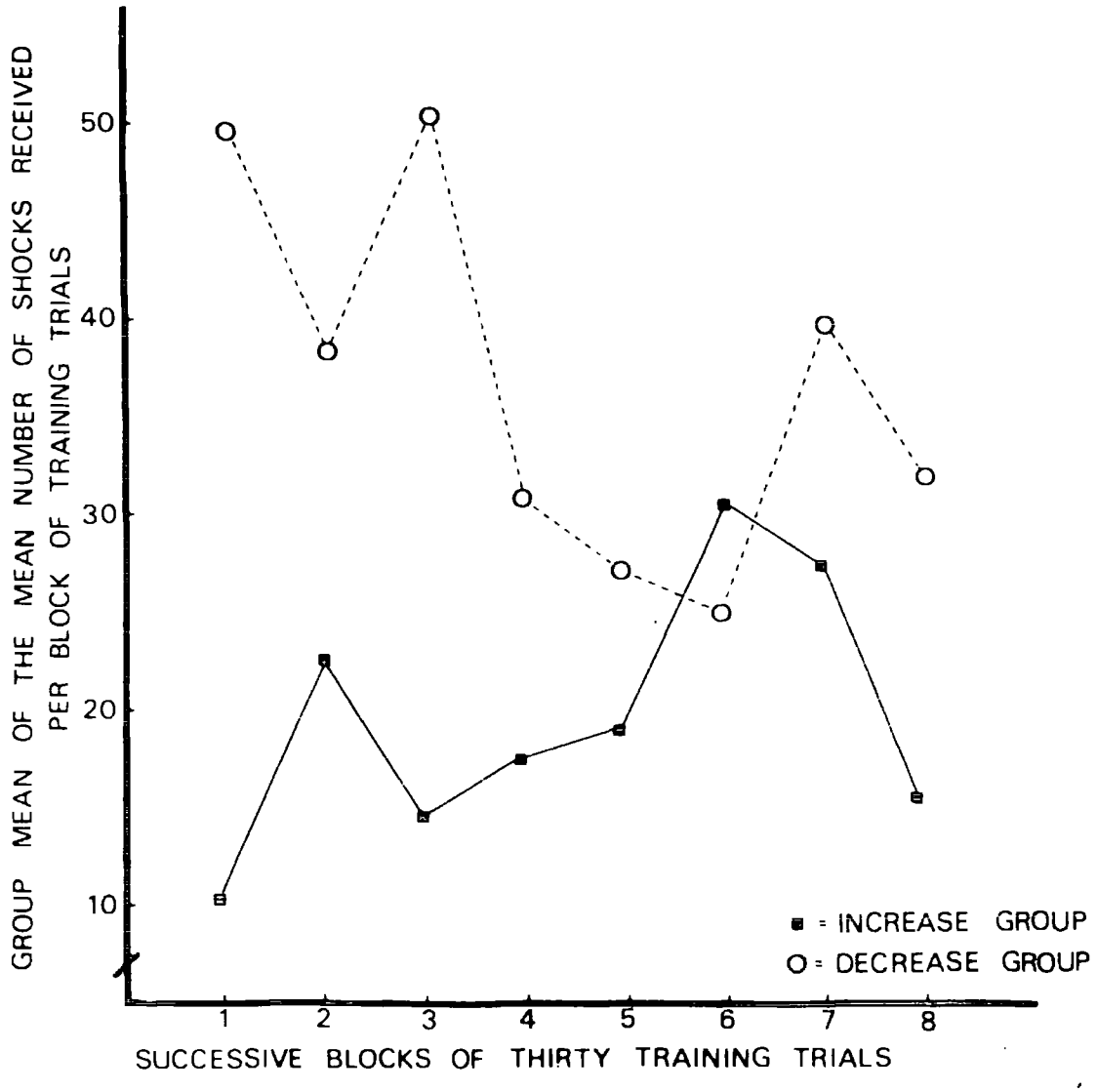

FIGURE 2.8 The change over successive blocks of trials in the group mean of the mean number of shocks received per block of ten operant trials for the groups of rats shaped for either increases or decreases in heart-rate. These changes indicate that the animals shaped for decreases in heart-rate received fewer shocks as training proceeded whilst, in contrast, the mean number of shocks received by animals shaped for increases in heart-rate remained fairly constant over the training period.

There was also an apparent difference between the two groups in the mean number of trials per block of ten operant trials in which at least one shock pulse was received. This can be seen in Figure 2.9 which presents a plot of this parameter, one of shock probability, and the percentage change in heart-rate over the training period, for each animal. This plot shows that the decrease group received shocks on fewer trials than the increase group. This difference occurred even though the decrease group animals received more shocks per block of trials than did animals in the increase group. The values of the mean number of trials per block of trials in which a shock was received is presented for each animal in Table 2.B. A comparison of the difference between the two groups gave a value $t = 3.63$, 10 df; $p > 0.01$, < 0.005.

DISCUSSION

Although the results of this experiment seem encouraging, there are several factors which posed questions as to the possible variables which may have influenced the particular heart-rate changes which were obtained.

The results were disappointing, in that there was little evidence for discrimination of heart-rate response levels between reinforced, non-reinforced and blank trials. However, certain aspects of the study were not conducive to the demonstration of discrimination learning. Firstly, the length of the training session may have been sufficient only to show differences between increase and decrease groups and secondly, the experimental procedure may have put the animals under considerable stress. Empirical justification for this hypothesis was obtained in a later experiment (Chapter Eight). This being the case, it is likely that the generalisation gradients to the stimuli in the situation were flat and if the degree of

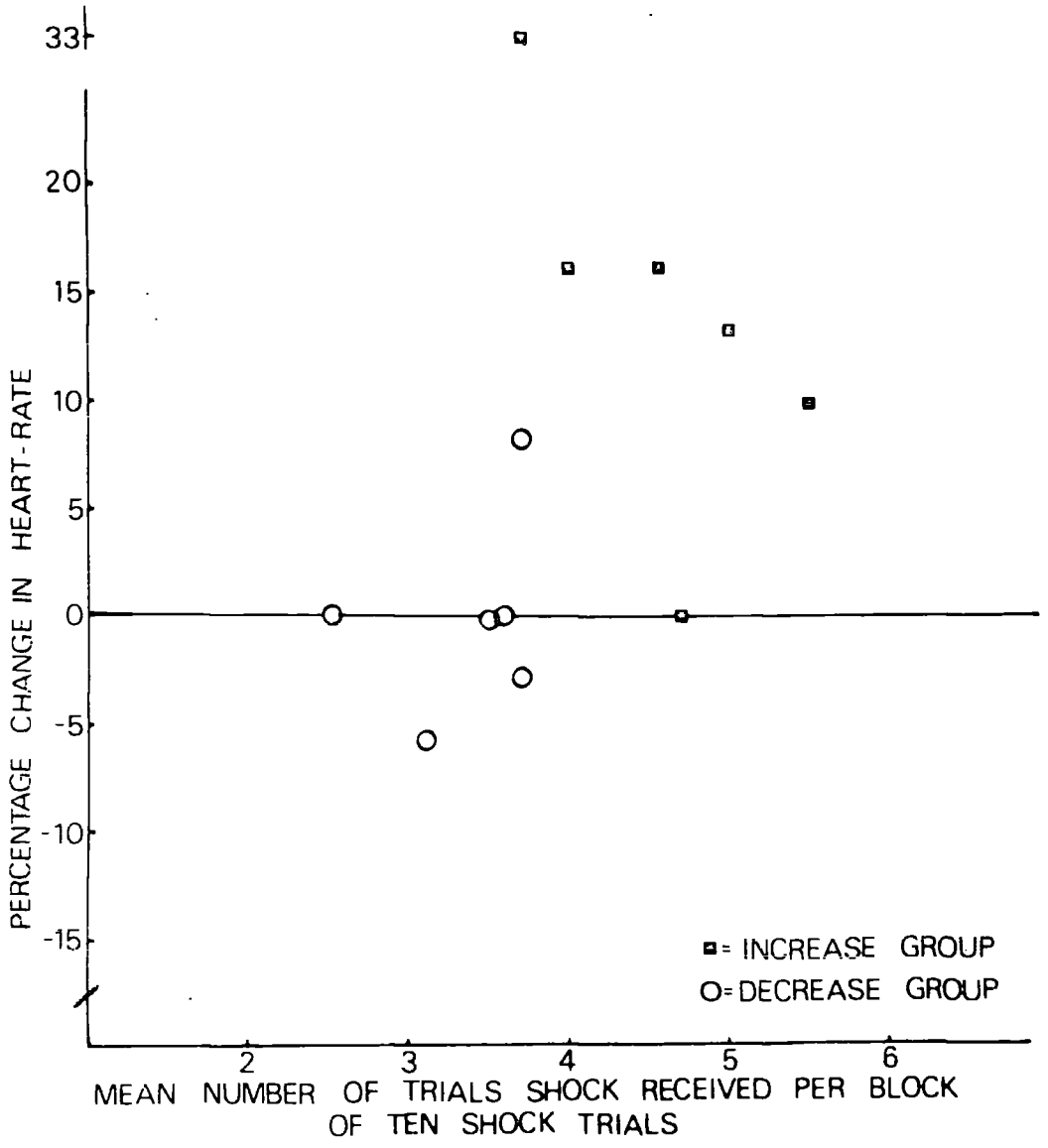

FIGURE 2.9 A plot of the mean number of trials per block of ten operant trials on which a shock was received by each animal, and the percentage change in heart-rate over the training period for that animal. The plot shows that rats shaped for heart-rate decreases received shocks on fewer trials than rats shaped for heart-rate increases.

generalisation was very high, then this would not enhance the probability of demonstrating discrimination in heart-rate responses between different types of trials.

There was also an apparent failure, in the experiment, to show a decrease in heart-rate response in animals shaped for that response (Figure 2.6). One possible explanation of this result (which is treated in some detail in Chapters Four and Six) is related to the problem of assessing the baseline heart-rate response in the artificially ventilated, curarised animal. It is sufficient here to note that the interpretation of the specific directional effects of operant schedules on the heart-rate response must be related to the baseline response rate to the schedule of stimulus and shock presentations in the absence of operant contingencies. This baseline rate was not recorded in the present experiment and, therefore, it cannot be concluded that the experiment necessarily demonstrates an inability to shape a decrease heart-rate response.

A second explanation is related to the possible blocking action of d-tubocurarine chloride on autonomic ganglia. There is evidence which suggests that there may be a selective blocking action on parasympathetic ganglia, with consequent vagal blockade. This effect would result in considerable difficulty in operant conditioning of heart-rate decreases. Support for this proposition is reviewed in Chapter Four, and experimental evidence is presented which would indicate that this blocking effect did not occur in the experiment which has been presented in this chapter.

A third explanation which could also be postulated, stresses the importance of the reinforcer used and its effect, per se, on the heart-rate response. Cardiotachometer recordings from two animals in the increase group and two animals in the decrease group, showed clearly that the unconditioned response to the first shock,

on any one trial, was always a slight tachycardia (Figure 2.10). Within any one CS+ trial, the response was consistent for the first few shocks but, as more shocks were received, the response became unstable (Figure 2.11) and, in many cases, there was apparent habituation of the unconditioned response. This effect suggests that the two groups were undergoing somewhat different training schedules. For negative reinforcement to have an effect, and either escape or avoidance occur, the appropriate response must first be produced. This occurred for the increase group but, for the decrease group, more shocks were received, as a consequence of the unconditioned tachycardia; that is, punishment was given for the production of the inappropriate heart-rate response.

This analysis not only explains why it may have been difficult to condition heart-rate decreases, but also illuminates the importance of the interaction of criterion changes and the effects of the reinforcer on the heart-rate response. In the decrease group animals, remedial changes of criterion level over training were frequent, whilst they were seldom required for animals in the increase group. In a similar way, it could be argued that the effect of pairing the stimulus and shock led to a classical conditioned component of heart-rate change. The direction of this component would be of importance in determining the success of operant conditioning of changes in heart-rate in a specific direction.

The effect of the unconditioned heart-rate increases to initial shocks on a CS+ trial increased the probability that animals in the decrease group would receive more than one shock on any such trial. As the shifts in criterion levels for the two groups were of similar magnitude, the effect was that animals in the decrease group received a greater mean number of shocks per block of trials (Figure 2.7). This complicates the interpretation of the results of the heart-rate changes obtained in the experiment. The differences between the

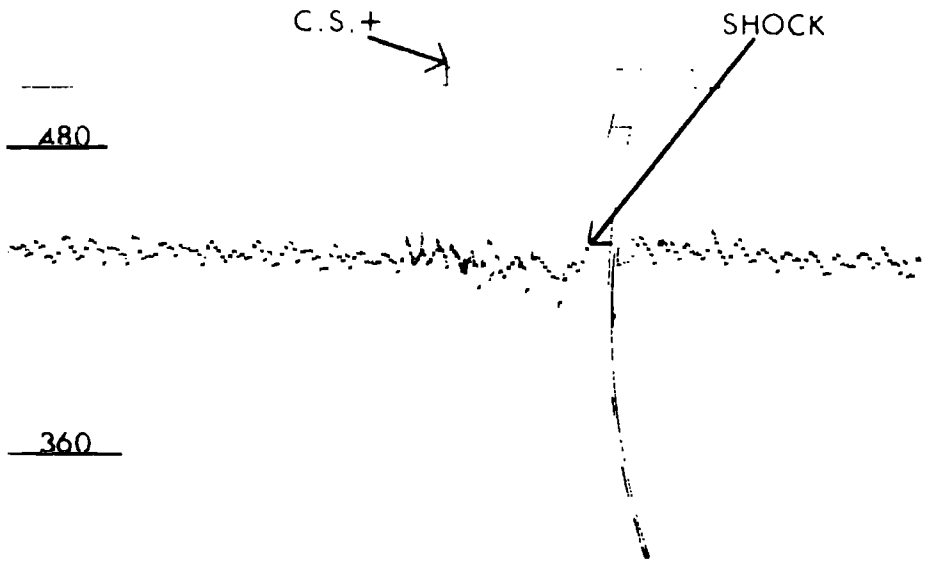

FIGURE 2.10 The cardiotachometer record, during an operant CS+ trial, for Rat Subject No. 2 shaped for increases in heart-rate. The unconditioned heart-rate response to shock was a mild tachycardia.

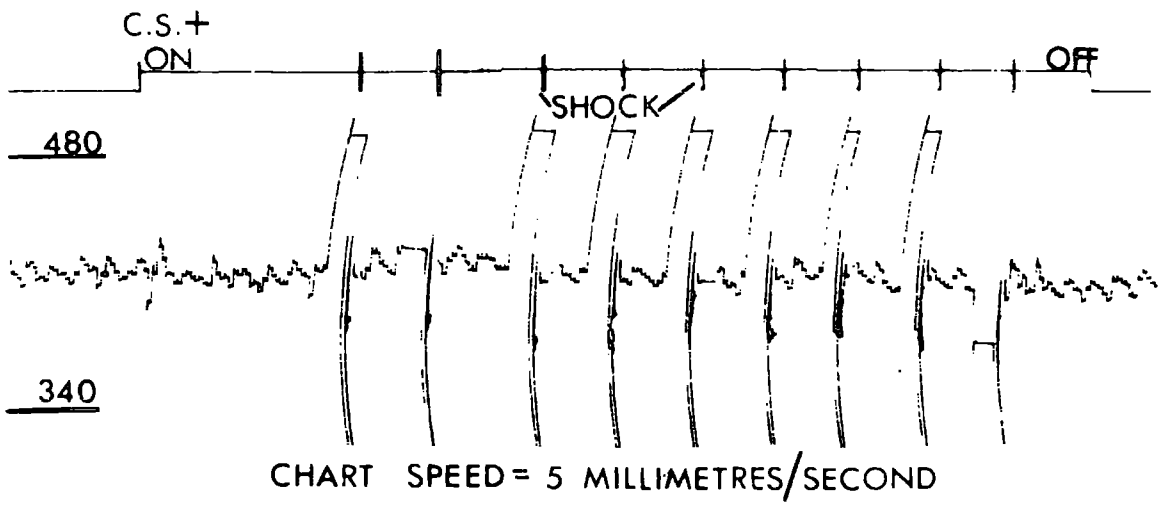

FIGURE 2.11 The cardiotachometer record, during an operant CS+ trial, for Rat Subject No. 1 shaped for decreases in heart-rate. The mild tachycardia to the first shock becomes less evident as further shocks are presented. Eventually the heart-rate decreases and escape occurs.

changes in the decrease and increase groups may have been merely a reflection of the differences in the frequencies of shock received by animals in the two groups. However, partial correlation coefficients which were obtained for the variables of percentage change in heart-rate over training, the mean number of shocks received per block of trials, and the mean number of trials on which a shock was received per block of trials, indicated no significant relationships between heart-rate changes and either shock parameter for animals in both the increase and the decrease groups. Moreover, the percentage increases which were produced in the heart-rate response by operant manipulations were far greater than the unconditioned responses to shock.

The problem of differences in shock parameters for the increase and decrease groups could possibly have been remedied by use of different criterion level changes for the two groups. Provided the heart-rate responses between the two groups were mutually exclusive then, in the use of the bidirectional design, the particular criterion level change which is used for any one subject is unimportant. However, this procedure would require considerable skill on the part of the experimenter in choosing criterion level changes which led to balance in the number of shocks received by animals in the two groups. By using programming apparatus, it was hoped to avoid such experimenter effects in these early studies of operant conditioning of heart-rate changes. However, the absolute percentage shifts in criterion levels were altered by the experimenter throughout training and the decrease in the mean number of shocks received by the decrease group over successive blocks of training trials, despite absence of a decrease in heart-rate response (Figures 2.6 and 2.8) would suggest an experimenter effect.

The study presented in this chapter was completed in 1968 and it suggested a number of factors which required further investigation. During the period of this preliminary investigation, a number of studies were published which also attempted to evaluate the possibility of operant conditioning of heart-rate changes. These experiments came, primarily, from two laboratories and were very similar in conception to the experiment presented above. Because of the complementary nature of these studies and because of the importance of their results to the content of this thesis, they are presented in some detail in the following chapter.

CHAPTER THREE

OPERANT CONDITIONING OF HEART-RATE CHANGES IN THE CURARISED ANIMAL - 2.

1. Operant Conditioning of Heart-Rate Changes in the Curarised Rat.

Trowill (1967) performed the first of a series of experiments at the Rockefeller University, New York, which attempted to show operant conditioning of an autonomic response. Curarised rats were used in these experiments and the autonomic response chosen was again heart-rate. The dose levels of d-tubocurarine used completely suppressed EMG recordings from the gastrocnemius muscle. The basic conception of the experiments was similar to that presented in the previous chapter. However, the reinforcer used was positive electrical stimulation with electrodes placed in the medial forebrain bundle of the lateral hypothalamus. In Trowill's experiment, the rats were initially trained to press for this intracranial self-stimulation in the non-curarised state. During operant heart-rate training, under d-tubocurarine paralysis, a criterion level of heart-rate was set so that when the heart-rate of the animal was above this preselected criterion level, the animal received stimulation. A bidirectional design was incorporated and, consequently, a second group of rats was used in which stimulation was given when the heart-rate of the rat dropped below a preselected criterion level. Predetermined levels were set so that it could be expected that the appropriate heart-rate response would be attained once every three to five seconds. The reward stimulation was delivered on an eight seconds fixed interval schedule. The results showed that fifteen of the nineteen rats reinforced for heart-rate increases, produced the appropriate response. Of the seventeen rats reinforced for decreasing heart-rates, fifteen

showed an apparent decrease in response. The difference between the groups was statistically significant. However, the results were confounded by the data obtained from further animals which had been yoked to animals in these experimental groups. The differences between experimental and yoked controls in heart-rate responses for animals in the increase group were smaller than the analogous differences for the decrease group. Furthermore, the frequency of correct response changed little for the increase group rats over the training period, but was highly significant for rats in the decrease group. This effect resulted in fewer stimulations being received by the increase group. The implication of the results was that greater success had been obtained in conditioning a slowing of heart-rate than in conditioning an increase.

Miller and DiCara (1967) performed a follow-up study in the same laboratories, using the same apparatus and a similar bidirectional design. They attempted to shape greater heart-rate changes in their curarised rats by progressive shift in the criterion to a more difficult level as conditioning proceeded, a procedure which was similar to the one described in the previous chapter. The initial criterion levels were selected so that they were expected to be reached in approximately five seconds. If, in the presence of an operant, CS+ stimulus, the criterion level was reached in approximately half that time then the level was made more difficult. However, if for a period of time, it took ten seconds to attain the criterion level, then a shift of two percent was made in the opposite direction. During a ninety minute training period, heart-rate changes as large as twenty percent from baseline rates were produced by the operant training procedure. These effects, and the differences between the

increase and the decrease groups, were not a consequence of the number of brain stimulations received, because both groups were found to have received an equivalent number of stimulations.

In Miller and DiCara's experiment, an attempt was made to show that the operant heart-rate response occurred only in the presence of the stimulus situation in which it was rewarded, rather than one in which it was not. This evidence of discrimination learning was not found but, if the animals were given a further ninety minutes of operant training, then evidence was obtained which indicated that the heart-rate response showed differential effects in the presence of a reinforced and a non-reinforced stimulus situation.

Miller and DiCara (1968a) performed a third experiment on operant conditioning of heart-rate changes in the curarised rat. Again a bidirectional design was used and progressive shaping of the appropriate responses in the heart-rate by two percent changes in the criterion levels was employed. Discrimination training was also incorporated into the experimental design using reinforced (CS+), non-reinforced (CS-) and blank trials in the same manner as the experiment presented in the previous chapter. The reinforcer used was pulsed shock of 0.3 mA delivered to the base of the tail. On CS+ trials, if predetermined heart-rate criterion level was achieved within five seconds, then no shock was delivered. If an animal failed to reach criterion level within this time, then it received a 0.1 second pulse shock and continued to receive shock approximately one every two seconds until the criterion level was attained. This procedure was exactly the same as that used in the preliminary study presented in the previous

chapter. Every tenth trial was again a test trial in which heart-rate was measured during five seconds of the operant, CS+ stimulus. However, in this study the test trial was followed by a normal CS+ trial without interrruption of the operant stimulus. During training, each block of ten operant trials was analysed and the criterion made more difficult if the animal changed its heart-rate sufficiently to enable the criterion level to be attained in not less than an average of three seconds. Similarly, the criterion level was made easier if the animal was taking, on average, more than ten seconds to meet the criterion level. The schedule was programmed so that the three types of trial were given in a counter-balanced sequence in which each combination of two trials, including two of the same kind, occurred an equal number of times in each cycle of eighteen trials. Avoidance training consisted of three hundred trials presented on a VI 30 seconds schedule. The results showed significant differences between the increase and decrease groups in the heart-rate responses. The increase group rats showed increases in the rate of about 15 per cent, and the decrease group rats, decreases of a similar magnitude. There were also differences in the heart-rate responses, between the different types of trials, for animals in both groups. Although the change in response was greatest during operant trials, there was considerable change in the same direction for the baseline responses. The numbers of shocks received by rats in the two groups were very similar and, consequently, it was not possible to attribute differences in heart-rate changes to the direct effects of shock.

2. Operant Conditioning of Heart-Rate Changes in the Curarised Dog.

In 1967, A. H. Black published an excellent report on his attempts to operantly condition heart-rate in the curarised dog. In his experiments, the level of curarisation was sufficient to prevent overt skeletal movement, but not of a level that suppressed EMG recordings; even so, artificial ventilation was required. Again, a bidirectional design was used, and the reinforcement was an electric shock of 4-10 mA intensity. However, a different principle for selecting criterion levels was used. The procedure for the dogs reinforced for heart-rate increases was described as follows:

"On each trial the maximum heart-rate during twenty seconds of the inter-trial interval was determined, before onset of a white noise (operant) stimulus (CS+). If heart-rate was maintained above this level for six seconds during the CS+ they were reinforced by termination of the white noise and shock avoidance. If this six second period had passed and the dogs failed to maintain criterion rate, they were given a brief shock following the second beat below criterion. Shock was followed by a four second dead procedure, and then the reinforcement procedure that followed CS+ onset was reinstituted. The procedure for operant conditioning of heart-rate decreases was the same, except that everything was turned upside down".

Results showed a significant increase in the number of avoidances over training for dogs in both increase and decrease groups. The criterion for termination of training was twenty consecutive avoidances. This criterion was achieved more quickly for dogs in the increase group. Cardiotachometer recordings indicated that the

appropriate heart-rate changes occurred only during presentation of the operant stimulus. Baseline response rate during inter-trial intervals was unaffected.

SUMMARY

The studies presented in this chapter again provide considerable positive evidence for operant conditioning of heart-rate changes. However, there are anomalies in the results of the various experiments. The discrepancy between results obtained in the experiment of DiCara and Miller (1968a) and those presented in the previous chapter is of considerable interest because the two experiments were very similar. The stimuli used, the negative reinforcer, the method of reinforcing heart-rate changes and assessment of criterion levels were almost identical. There were, however, differences in the schedule of trial presentations and the method for determining when changes in criterion levels during training should occur. Differences in the results can be related to:-

(i) The heart-rate changes which were obtained.

(ii) The number of shocks received by each of the two groups.

(i) The heart-rate changes obtained in experiments on operant conditioning of heart-rate changes in curarised animals.

Unlike the experiment presented in the previous chapter, the results of the other experiments, which have been presented above, provide good evidence for discrimination learning in heart-rate changes. It is just to comment, that in the experiment of Miller and DiCara (1967), using electrical brain stimulation as a reinforcer, considerable difficulty was encountered in showing discrimination

learning. However, their later experiment, Miller and DiCara (1968a) in which shock was used as the reinforcer, provided excellent evidence for discrimination of heart-rate response between the various trials even during early stages of the training schedule. The experiment performed by Black (1967) is alone in demonstrating that the appropriate heart-rate response, and that response only, occurs during the presence of the operant, CS+ stimulus; moreover, the response was shown not to occur in other, time-out conditions.

The demonstration of operant discrimination learning of the heart-rate response would be a valuable adjunct to the initial studies, since it would imply that similar processes are in operation to those which apply during operant conditioning of skeletal responses. The effect of these studies, whilst encouraging, is to leave the problem unresolved. What is required is a parametric study in which the initial experimental conditions are those most conducive to success in discrimination learning.

The results of the experiments presented in this chapter complement some of the suggestions which were made in the previous chapter as to the various factors which may influence the specific direction of heart-rate changes obtained. One such factor was suggested to be the unconditioned effects of the reinforcer. In Trowill's (1967) experiment, in which electrical brain stimulation was used as a positive reinforcer, it was found that heart-rate decreases were more easily shaped than heart-rate increases. Although no observations were described in that experiment, Hothersall and Brener (1969) showed that positive brain stimulation, similar to that used in Trowill's experiment, resulted in an unconditioned bradycardia.

Black's (1967) experiment used a design which eliminated the direct effects of the unconditioned response to the reinforcer. This was accomplished by the use of a dead period after each presentation of the reinforcer during which the operant schedule was not in effect. The results showed success in shaping both increases and decreases in heart-rate. This suggested the effects of tachycardia, produced by the shock reinforcement, did not interfere with the operant conditioning of the heart-rate decreases. However, in Black's experiment, a lower criterion level was used for the heart-rate decrease group. Since success of conditioning was measured in terms of number of shock avoidances, it is difficult to ascertain whether the relative success in shaping decreases in heart-rate was due to the lowered criterion level, the use of the time-out interval, or an interaction of these factors.

(ii) The numbers of reinforcements received by the increase and decrease groups in experiments on operant conditioning of heart-rate changes in curarised animals.

It was suggested in the previous chapter that not only were problems of shaping specific changes in heart-rate responses related to the effects of the unconditioned response, but also that this effect could subsequently cause differences in the number of reinforcements received by the two groups during training. The problem, of course, does not arise in Black's experiment as the dead period eliminates such effects. However, it does not account for the results of the experiments performed at Rockefeller University, in which overlapping numbers of reinforcements were claimed to be received by the animals in both the increase and decrease groups.

Trowill's results are an exception, and show the expected differences between the groups in the number of reinforcements received by each group. In DiCara and Miller's (1968a) study, the claim for equality in the number of shocks received was substantiated by their data for only the last block of training trials. The data for the previous trials is not given. Assuming that this block was representative of the remainder, then it is possible that the experimenters were fortunate in choosing a schedule, parameters of reinforcement and stimuli which, for their animals, led to this overlap in shock frequency received by the two groups. If success in demonstrating the validity of operant conditioning of autonomic response is restricted to narrow experimental limits, then the principle would lose much of its importance and attraction. It is hoped to demonstrate in this thesis that operant components are of fundamental importance in most studies of autonomic conditioning.

CHAPTER FOUR

THE CURARISED RAT

Although curare paralysis is a convenient technique for ruling out the possibility of skeletal mediation of autonomic responses, there are certain considerations which should be borne in mind when using curarised animals. There are two sets of practical considerations involved in the studies on operant conditioning of heart-rate changes which are related to the use of curare. One problem is concerned with the requirements of artificial ventilation by the curarised animal. The second problem arises from a consideration of the pharmacological and physiological action of curariform drugs.

PROBLEMS IN ARTIFICIAL VENTILATION OF THE CURARISED ANIMAL

The problems involved in, and the effects of, artificial ventilation are very relevant for experiments on operant conditioning of heart-rate responses. Ideally, it would be desirable to continuously monitor the partial pressures of oxygen and carbon dioxide in the blood, to ensure that values were stable throughout the training period. Unfortunately, these technical resources were not available for the experiments presented in this thesis. However, it is significant that no study using curarised animals has yet taken the precaution of monitoring the effects of artificial ventilation in this manner. Consequently, the possibilities of hyperventilation and hypoventilation are real. Briefly, hyperventilation results in washing out carbon dioxide from the lungs, with a consequent effect on the vasomotor centre in the brain stem. The result is a fall in blood pressure and, therefore, reflex effects tending to increase heart-rate. Hypoventilation produces a similar action on the vasomotor centre through the lack of oxygen, both directly, and indirectly, by action of the chemoreceptors. The

possible effects of ventilation become complex when there is also excess carbon dioxide in hypoventilation, for this accentuates the effect of the oxygen deficit on the vasomotor centre. The problem is, however, even more complex since increase in carbon dioxide content has various effects. Green (1965) has pointed out that carbon dioxide acts centrally as a vasoconstrictor and yet peripherally as a vasodilator. Gellhorn (1953) demonstrated that inhalation of excess carbon dioxide increases hypothalamic excitability with consequent effects on blood pressure. These effects on blood pressure changes produce reflex changes in heart-rate. Hence, the conditions of artificial ventilation over the training period must be stable, in order to give a stable baseline heart-rate response and, therefore, subsequent changes in response level due to operant training techniques may be precisely assessed.

Two mechanisms of artificial ventilation are available for use with the small mammal and both types were used in the experiments reported in this thesis. Both methods use positive pressure systems which have a disadvantage in that the pressure gradient within the trachea, bronchi and bronchioles is such that it is considerably greater at, and tends to act from, the points furthest away from the alveolar sacs where gaseous exchange occurs. One other effect of positive pressure systems is that they cause disturbances in circulatory changes similar to valsalva manoeuvres, but smaller in nature. The result is a lowered cardiac output and disturbance in the phase relationship of blood pressure and rhythmic changes in venous return, effects which have been discussed by Levy, De Geest and Zieske (1966).

The two types of positive pressure system which have been used differ primarily in that one has a passive expiration stroke and the other an active expiration stroke.

Type 1. Positive Pressure Ventilation with Passive Expiration

Using this respirator, positive pressure is applied for inspiration and then the lungs are allowed to collapse under their own elasticity when the pressure is cut off by a valve which also provides an outlet system for the expiration. Early attempts at ventilation using this type of pump proved extremely difficult as the baseline heart-rate rarely became steady, or stable, over time. Also, a number of animals died under these early attempts. Varying the volume and pressure of the inspired air, did not result in increased success.

As a result of these failures, it was decided to investigate the possibility of changes in oxygen and carbon dioxide content of the blood during the conditions of ventilation. Small blood samples were taken by heart puncture from three artificially ventilated, anaesthetised, curarised rats, at fifteen minute intervals from the start of ventilation. The technique used to obtain these samples has been reported by Creskoff, Fitz-Hugh and Farris (1963). A small portion of each blood sample was taken from the heparinised syringe into a fine heparinised capillary tube, a small bore magnet inserted, and the tube sealed with wax. Each sample was kept continually stirred by the magnet and rushed to a chemical pathology laboratory for analysis. The results showed that there was a progressive increase in carbon dioxide content and a decrease in oxygen content in successive samples from each animal. The results could be summarised by a diagnosis of hypoxia and hypercapnia. The cause of this appeared to be the amount of 'dead space' within the ventilation system, since the increases in the inspiratory volume did not produce any dramatic improvement. The problem was accentuated by ventilating through a snout mask rather than by a tracheal cannula, as the mask increases the 'dead space' by a considerable factor. The solution to the problem was obtained by metering an increasing proportion of oxygen into the

inspired volume until a stable baseline heart-rate response was obtained and further analyses of blood samples showed an absence of both hypoxia and hypercapnia. An excellent overt index of the correct conditions is seen in the colour of the hind feet. When ventilation is satisfactory, the hind feet appear a healthy 'pink-white' colour, under incorrect ventilation conditions they appear a 'very white-blue colour'.

Another factor, which prevented adequate ventilation on some occasions, was constriction of the nasal passages. This occurred if the balloon used for the snout mask had limited elasticity. The problem was eliminated by stretching the neck of the balloon over a boiling tube for a few days before its use as a snout mask.

The formation of mucous in the respiratory passages poses yet another problem in artificial ventilation. Endotracheal intubation with subsequent suctioning was not used, since it is known to produce cardiac arrhythmias (Shim, Fine, Fernandez and Williams, 1969). A solution was found by tilting the experimental animal slightly forward, so allowing the mucous (and saliva) to drain from the mouth. This procedure is imperative, since swallowing cannot occur under curare paralysis. Also, Corbett (1952) has shown an increase in the rate of salivation in curarised dogs, which he assumed was due to central nervous system effects.

One final problem which affects artificial ventilation and, consequently, baseline heart-rate is related to a side effect which is produced by d-tubocurarine. This drug causes a release of histamine which often causes bronchoconstriction and, in some animals which are very sensitive to histamine (e.g. guinea pigs), this may be fatal. The effects of bronchoconstriction are to produce changes in the tidal air flow with change in the content of oxygen and carbon dioxide in the blood. This then produces reflex changes in blood pressure and, consequently, in heart-rate.

Type 2. Artificial Ventilation With Active Expiration

The second type of positive pressure pump used was commercially available (E. & M. Instruments Ltd.) and has an active expiration stroke in which air is drawn from the lungs. Although many of the problems of artificial ventilation, which have been elaborated above, also apply when this pump is used, it has two great advantages. To obtain a stable baseline heart-rate response it was not necessary to add oxygen to the inspired air. Also, the ratio of the time of the inspiration stroke to the time of the expiration stroke could be accurately varied. In the pump with a passive expiration stroke, previously described, there was a relatively slow steady pressure atinspiration, followed by a sharp reduction to zero pressure at expiration. The net effect was to facilitate movement of mucous to the extreme bronchioles, with consequent reduction in the effectiveness of ventilation. However, with the active expiration pump, this movement of mucous could be prevented by balancing the ratio of inspiration to expiration. Despite this advantage, the precaution of tilting the animal slightly forward, to allow for drainage of mucous and saliva, was still observed.

The experiments of Miller and his associates, take an approach which seems to disregard some of the inherent dangers in artificial ventilation. In an attempt to overcome problems related to differences between animals in their initial baseline levels (see Wilder, 1956) the depth of ventilation was varied so as to produce heart-rates of approximately the same level in each animal during the adaptation period before operant training. This is not only dangerous for suitable ventilation, but also stems from a misinterpretation of the Law of Initial Values. This law, which governs the relation of response change to baseline rate, emphasises

changes in heart-rate within one individual. Since individuals are known to have differing heart-rates under normal conditions, the attempts at control used by Miller and associates would seem inappropriate. In the experiments presented in this thesis, rats of almost identical body weights were used in the increase and decrease groups, and they were ventilated under constant conditions. Moreover, animals in the two groups were matched as closely as possible for their initial baseline heart-rates which were observed in the thirty minute adaptation period.

One other interesting comment which applies to the effects of artificial ventilation, is the ambiguity which could have arisen from the use of the term stabilisation, with reference to the heart-rate response. Stabilisation could mean that the baseline conditions of artificial ventilation which were used, gave a low variance in the inter-beat-intervals over short periods of time, or it could mean that a constant heart-rate response was obtained over a period of about three hours. Only in a recent article does Miller (1969) clarify this point for his own conditions of ventilation. In this article he confirms that, not only does the variance in the inter-beat-intervals become much reduced in the adaptation period, but also that the baseline heart-rate response is steady during time-out conditions, for periods equivalent to the length of the training sessions. In the present series of experiments, heart-rate records were obtained from a cardiotachometer for several animals under baseline conditions of artificial ventilation and curarisation, using both types of respirator. For the pump with passive expiration, this was found to be constant for periods of up to three hours. However, using the pump with active expiration, a slight mean decrease in the heart-rate response of approximately two percent was found during a similar interval under the baseline conditions.

These problems, associated with the conditions of artificial ventilation, are unlikely to affect the results of the experiments presented in the previous chapters because the factors should be balanced by use of the bidirectional design and, consequently, a real difference in heart-rate responses between the groups will be greater than variations due to the conditions of ventilation. However, they do show the importance of assessing the heart-rate response under baseline conditions of ventilation and curarisation.

Many of these criticisms which have been elaborated do not apply to the results of the experiment performed by Black (1967). In that experiment, the heart-rate responses, during the operant conditioning trials, were measured in relation to the baseline heart-rate response, rather than by changes in the baseline rate of responding over successive trials. Moreover, the artificial ventilation was applied via a tracheal cannula which considerably reduces the problems caused by 'dead-space' within the respiratory system and which is an easier and more satisfactory procedure for the dog than the rat.

Hahn (1971) has recently described his technique and procedure for artificial ventilation of the curarised rat. The procedure and his comments are very similar to those described above; however, he makes one interesting additional contribution in suggesting that adequate ventilation can be inferred directly from the heart-rate itself. This should be between 350 and 420 bpm, with variation of 20 bpm, under baseline conditions: if the baseline rate is higher than 460 bpm, then he suggests there is a danger of hyperoxemia and the heart-rate becomes locked-in and insensitive to stimulation. It should be noted that these figures are for rats weighing between 400 and 600 gm. and it is likely that rates are higher for smaller animals under satisfactory conditions of ventilation. In the present series of

studies, baseline heart-rates were between 396 and 486 bpm for rats weighing 200 to 300 gm., which are slightly higher than the values suggested by Hahn, but were similar to the baseline rates of animals in the studies of DiCara (1970) and Hothersall and Brener (1969).

THE PHARMACOLOGICAL AND PHYSIOLOGICAL ACTION OF D-TUBOCURARINE CHLORIDE

Curare is the generic term for various South American Indian arrow poisons which may contain several different alkaloids. Early research work with these drugs was related to the mechanism of transmission of nerve impulses between the nerve and skeletal muscle. In animals paralysed with curare, stimulation of the motor nerve does not excite twitch responses in the muscle it innervates. Curare does not block conduction of nerve impulses in nerve fibures and direct electrical stimulation of a muscle can produce muscle twitch. Bernard (1856) concluded that the site of curare blocking action is at the junction between the nerve and muscle. He also demonstrated that curare in excess of that required to paralyse skeletal muscle end-plates, blocked the cardio-inhibitory fibres of the vagus nerve, and concluded that curare paralysed motor nerve endings in unstriated muscle in a similar manner to its effect on striated muscle. This conclusion was disputed by Dickenson and Langley (1890) who believed the action of curare to be on autonomic nerve cells or ganglia and demonstrated this action on the sympathetic cervical ganglion of the rabbit. Langley (1918) suggested a much wider blocking action by curare in the preganglionic nerves of the cat, and showed that different ganglia were differentially sensitive to curare.

The doses of d-tubocurarine chloride which were used in every experiment (except that of Black, 1967) on operant conditioning of heart-rate changes which have been reported in previous chapters, were sufficient to suppress not only overt muscular activity, but also

all electromyographic recordings. These high dose levels were administered to completely exclude the possibility of proprioceptive feedback from ~~gamma motor neurons~~ muscle spindle receptors. Smith (1964) and Black (1966) suggested, and experimentally demonstrated, that such feedback was sufficient to enable operant conditioning of covert skeletal responses to be effected and these responses were shown to produce heart-rate changes. Black (1967) found operant heart-rate conditioning impossible to demonstrate in the curarised dog when dose levels were used which were sufficient to completely suppress electromyographic (EMG) recordings, although producing the most convincing evidence for the phenomenon at dose levels sufficient to suppress only overt skeletal movement. Black interpreted differences in his results, at different dose levels, as being caused by the ability of d-tubocurarine chloride to block neuronal transmission in autonomic ganglia at dosages which were sufficient to completely block the neuromuscular junction. This explanation was preferred to the conclusion that operant heart-rate conditioning per se did not occur because, as dosage was increased, a previously conditioned operant heart-rate response became unstable before the EMG was suppressed. If Black's interpretation is correct, it raises a considerable question as to the validity of other experiments which have used drug dosages sufficient to completely suppress EMG. The contradiction in views is acute since DiCara (1970) has claimed that d-tubocurarine chloride has no blocking action on autonomic ganglia. However, he does not provide any direct experimental support for his statement. These conflicting points of view question the suitability of the use of the curarised animal in studies of autonomic conditioning. They also suggest that further investigation into the properties of curariform drugs, in particular d-tubocurarine chloride, is required.

The effects of d-tubocurarine on the central nervous system.

Unfortunately, curare was found to have some anaesthetic type effects on the central nervous system. However, King (1935) isolated a pure

substance tubocurarine and a study of its structure showed it to contain two quaternary nitrogen atoms separated by ten other atoms (Figure 4.1). A dextro rotatory isomer d-tubocurarine is now synthesised commercially. The structure is similar to that of acetylcholine and it appears that the quaternary ammonium ions are of considerable importance for the blocking effects (Marshall, 1968). Smith, Brown, Toman and Goodman (1947) have reported that the EEG and the verbal report of the human subject of his memory and sensations under deep curarisation requiring artificial ventilation, are both undisturbed. This is because the pure synthetic product does not penetrate the blood-brain barrier (Riker and Wescoe, 1951) and should, therefore, have no direct effect on the central nervous system (Paton, 1959). However, McIntyre, Bennet and Hamilton (1951) have pointed out that the absence of effect on the central nervous system by d-tubocurarine depends on the dose and the route of application. The review of the evidence suggests that d-tubocurarine is capable of modifying central nervous system activity when large doses are given intra-arterially, whilst lower doses, administered at other sites may have no effect. These effects are probably due to the loss of sensory input from absence of movement and from gamma motor neuron activation of muscle spindles (Buchwald, Standish, Eldred and Halas, 1964). The loss of gamma motor neuron-proprioceptive feedback occurs only when doses are administered which are sufficient to suppress EMG activity.

The peripheral blocking effects of d-tubocurarine

Using an isolated nerve muscle preparation, Dale, Feldberg and Vogt (1936) demonstrated that the transmitter substance at the neuromuscular junction was acetylcholine. Ochs (1965) has summarised the evidence which shows that the same substance, acetylcholine, is involved in neural transmission in autonomic ganglia. In the Dale et al. article, it was shown that tubocurarine prevented neuromuscular

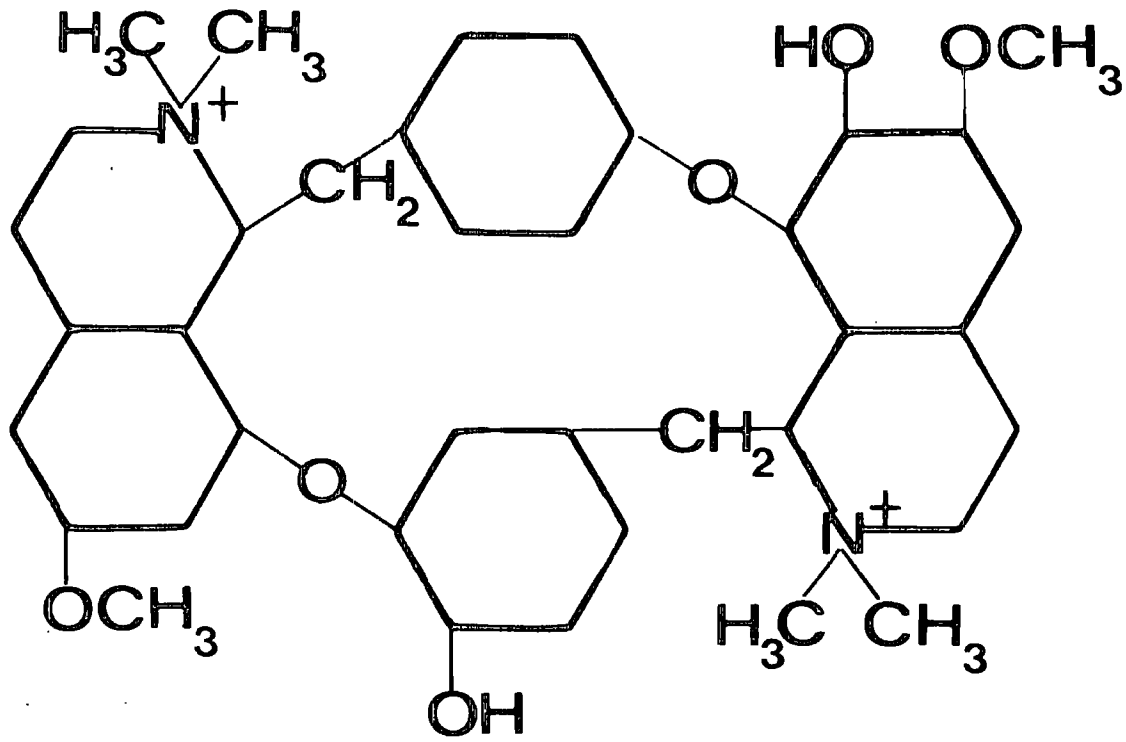

FIGURE 4.1 The structure of d-tubocurarine.

transmission without inhibiting the release of acetylcholine from motor nerve endings. Nachmansohn (1952) confirmed this and further indicated that the block at the neuromsucular junction, or in autonomic ganglia, by tubocurarine is produced without causing depolarisation of the postsynaptic membrane, but is caused by competition between tubocuarine and acetylcholine for protein receptor sites. Beani, Bianchi and Ledda (1964) have questioned these results and claimed to have shown that, under certain conditions, d-tubocurarine inhibits the release of acetylcholine from nerve endings. Chang, Cheng and Chen (1967) were unable to replicate the Beani et al results and could not, therefore, support their conclusion. Although other articles (see Marshall, 1968) have suggested that tubocurarine may inhibit the synthesis or release of acetylcholine in nerve endings, it is accepted that this effect is of secondary importance to its effect as a competitive blocking agent (Ochs, 1965).

A detailed investigation on the blocking effect of d-tubocurarine on the sympathetic ganglia of the rabbit by Eccles and Libet (1964), showed a competitive action with acetylcholine for those receptor sites at the postganglionic membrane which gives specific action to nicotine type agents. No detailed work has been presented for the effects of d-tubocurarine on parasympathetic ganglia, presumably because these are scattered throughout the end organ which they innervate and only in rare instances can they be recognised as discrete ganglia. Perry (1957), in a very brief summary of the literature on parasympathetic ganglia, concluded that the transmitter substance is acetylcholine and concludes, therefore, that there is little difference between the fundamental reactions in parasympathetic and sympathetic ganglia. Consequently, the search for differential blocking activities of drugs for these ganglia is unlikely to occur. The implication that curariform drugs affect parasympathetic response is

verified by early studies of Langley (1918), which show that curare can block the cardio-inhibitory fibres of the vagus nerve.

In summary, the evidence which has been accumulated from a variety of species, with diverse preparations and techniques, would suggest that d-tubocurarine, at high dose levels, produces a blocking effect in autonomic ganglia. The results of other experiments (Langley, 1918; Waser, 1959) would suggest that the degree of autonomic blockade was different in different ganglia, both within and across species, at different dose levels.

The crucial parameter for studies of operant conditioning of heart-rate changes would appear to be dose level. To satisfy theoretical principles for these more behavioural studies, it is necessary to completely suppress EMG activity. On the other hand, the dose must not be so great as to produce blocking effects in autonomic ganglia.

This chapter presents two attempts which were made at overcoming the problem of autonomic blockade by d-tubocurarine chloride.

1. Gallamine Triethiodide (Flaxedil): An alternative neuromscular blocking agent

Firstly, an attempt was made to find an alternative drug to d-tubocurarine which can completely suppress EMG activity but has little effect on autonomic ganglia. There are several possible drugs which are in common surgical use, and which produce effective neuromuscular blockade. Table 4.A presents a list of possible alternatives. The most suitable of these appeared to be gallamine triethiodide, and this was the one which was investigated for possible use in studies of operant conditioning of heart-rate changes. This drug produces a non-depolarisation block of the neuromuscular junction, similar to d-tubocurarine, but has been reported to have less effect

TABLE 4.A Drugs which produce neuromuscular blockade and which are used during surgical Anaesthesia.

Name	Type of Block	Duration	Histamine Release	Action On Autonomic Ganglia
1. D.tubocurarine chloride	Non-depolarization block of motor endplates	Prolonged	Marked	Blockade common
2. Dimethyl tubocurarine chloride	"	Prolonged	Less marked	Less blockade
3. Toxiferine I	"	Prolonged	Unknown	Unknown
4. Gallamine triethiodide	"	Prolonged	Very Little	Very Little
5. Suxamethonium chloride	Depolarization block	Very short	Slight	Slight stimulation
6. Suxethonium	"	Very short	Slight	Slight stimulation
7. Carbolonium bromide	"	Prolonged	Slight	Little or no effect

Information for this table was obtained from: 'Textbook of Pharmacology'. Bowman, W.C., Rand, W.J. Blackwell, Oxford. 1968.

on histamine release and autonomic ganglia. Unfortunately, preliminary investigations showed that dose levels of flaxedil, which were sufficient to completely suppress EMG activity, also produced very high baseline heart-rate levels. This made the drug completely unsuitable for experiments on operant conditioning of heart-rate changes in consequence of Wilder's (1956) Law of Initial Values which states that the magnitude of a change in an autonomic response to a stimulus is related to the pre-stimulus level. To reiterate this principle in Wilder's words:

"Not only the intensity but also the direction of a response of body function to any agent depend to a large degree on the initial level of that function at the start of the experiment. The higher this 'initial level' the smaller is the response to the function-raising, the greater is the response to the function-depressing agents. At more extreme levels there is a tendency to 'no response' and to paradoxic reactions i.e. a reversal of the usual direction of response".

The high resting heart-rate levels produced by flaxedil would make interpretation of heart-rate changes, produced in animals shaped for operant heart-rate increases, almost impossible. Moreover, the high levels of response suggest that the problem of ganglionic blockade is no less acute for the drug flaxedil than it was for d-tubocurarine, a contrast to the suggested effects during its use with surgical anaesthesia.

2. The blocking activity of d-tubocurarine on autonomic ganglia assessed by the effects of direct stimulation to preganglionic nerves.

The particular interest of this study was focused on those nerves controlling the heart. The difficulty in operant conditioning of a heart-rate decrease (see Chapter 2), in conjunction with the physiological and pharmacological evidence presented in this chapter, suggested a blocking effect on vagal nerve activity by d-tubocurarine at doses of 0.6mg./Kgm. body weight when injected intraperitoneally. This is the

dose level used in all the experiments presented in this thesis. The hypothesis was tested by stimulating the vagus nerve of the anaesthetised rat, both before and after injections of d-tubocurarine chloride, and noting the effects on the heart-rate response.

METHOD

Subjects

Four hooded rats, three males and one female, weighing between 200-280gm. were used. The rats were obtained from the Department of Psychology, University of Durham.

Apparatus and recording instrumentation

The nerve was to be stimulated by a bipolar electrode. Each electrode was made from quarter millimetre diameter, straight tungsten wire which was sharpened electrically using Hubel's (1951) technique. The electrode was insulated with Isonel 31 varnish (Schenectady Varnish Co., New York) to within a half millimetre of the tip by a procedure similar to that used by Marg (1964).

Electrodes for recording heart-rate were stainless steel needle electrodes which were inserted subdermally on either side of the thorax. The indifferent electrode was also a needle electrode inserted subdermally in the abdomen. The EKG signals were fed into a Beckman Type R.P. dynograph and a direct cardiotachometer reading gave the heart-rate in beats per minute.

Procedure

Three of the rats were deeply anaesthetised with Nembutal to a level that just suppressed respiration. The dose required was found to be approximately 45mg./Kgm. body weight injected intraperitoneally. The animal was then artificially ventilated with a small, positive pressure respirator (E & M Instrument Co. Ltd.) at sixty cycles per minute at a peak pressure of 10cm. of water and with a 1:1 inspiration to expiration ratio. Ventilation was applied via the snout mask made

from the neck of a rubber balloon.

When fully anaesthetised, the animal was turned on its back and the vagus nerve exposed on one side of the neck by dissection under a low power binocular microscope. After dissecting out and cleaning the nerve, it was prevented from drying out, and insulated from the surrounding tissue, by a covering of liquid paraffin which had been warmed to the body temperature of the rat. The vagus was then suspended over fine, plastic coated forceps and the bipolar electrode pushed into the nerve using a micromanipulator. Care was required in these operations to prevent the nerve from being pinched or stretched.

Stimulation to the nerve was delivered from a constant current stimulator at thirty-two cycles per second, at an intensity of 0.08mA for periods ranging from one to three seconds, at five minutes intervals. After the second period of stimulation, an intraperitoneal injection of d-tubocurarine chloride was administered at a dose level of 0.6mg/Kgm body weight. The effects of nerve stimulation on the heart-rate response, both before and after injection of d-tubocurarine, were noted from the direct cardiotachometer records of the experiment.

The procedure for the fourth animal was essentially the same, but the d-tubocurarine was injected fifteen minutes before commencing the dissection of the nerve.

RESULTS

All four animals showed evidence of a decrease in heart-rate during periods of stimulation of the vagus nerve, both before and after injection of d-tubocurarine chloride. The deceleration appeared to be dependent upon the duration of the applied stimulation (see Figure 4.2) and the effect is seen clearly on the cardiotachometer records because the baseline rate was extremely steady. Table 4.B shows the maximum decreases in heart-rate produced in each animal, during each period of stimulation and although these decreases were small, they were consistent.

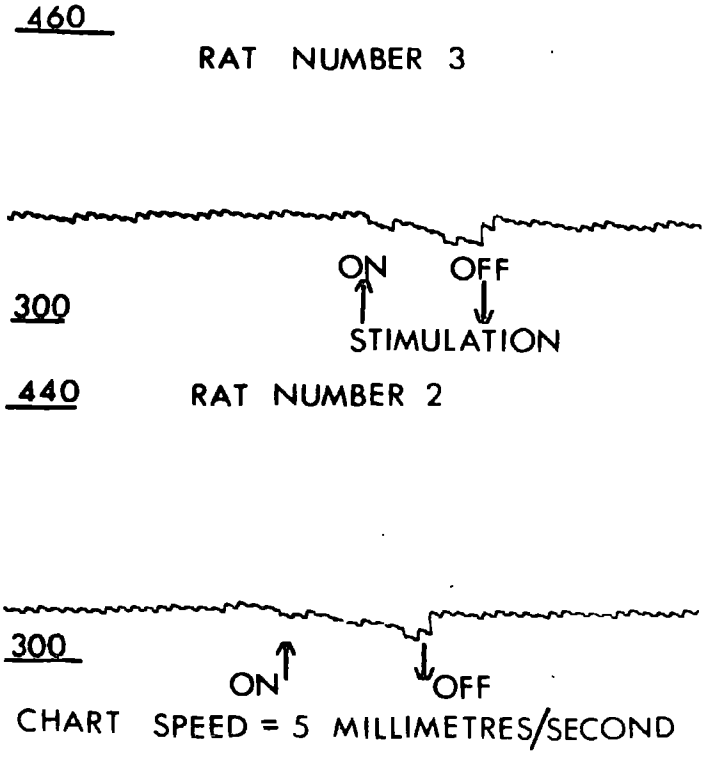

FIGURE 4.2 Examples of the effect of vagal stimulation on the cardiotachometer records of the heart-rate responses from two deeply anaesthetised, curarised rats. The bradycardia continued as long as the stimulation was maintained. The effect is consistently and easily observed because of the extremely steady baseline heart-rate of this preparation of the rat.

TABLE 4.B The effects of vagal stimulation on the baseline heart-rate response of the anaesthetised rat, both before and after an i.p. injection of 0.6mg/Kgm body weight of d-tubocurarine chloride.

RAT NO.	HEART-RATE CHANGE IN BEATS PER MINUTE stimulation periods						BASE RATE
	1	2	3	4	5	6	
1	5	7·5	18	6	4	12	340
2	10	7·5	6	10	8	12	325
3	12	8	8	12	16	8	360
4	10	10	13	7	9	10	320

vagus exposed

drug

drug

In each animal the effects of vagal stimulation were absent after approximately thirty minutes. This attenuation was unlikely to be produced by the blocking action of d-tubocurarine, because this effect should have been evident well before thirty minutes. The most likely explanation is that death of the nerve had occurred after that interval. This suggestion is supported by the data obtained from the fourth animal, since the effects of vagal stimulation on heart-rate were also absent in this animal approximately thirty minutes after exposing the nerve, even though d-tubocurarine was administered before starting the dissection.

DISCUSSION

The results indicate that d-tubocurarine chloride does not block the vagal parasympathetic effects to the heart of the dose levels used in the experiments presented in this thesis. This suggests that d-tubocurarine, at these dose levels, does not interfere with parasympathetic ganglionic transmission in the rat, and is a conclusion directly opposed to the results of the only other experiment which has attempted to assess the ganglionic blocking effects of d-tubocurarine at dose levels which were used in a study of operant conditioning of heart-rate changes. This experiment was reported by Black (1967), and again assessed the blocking effect of d-tubocurarine by direct electrical stimulation of cardiac nerves under increasing drug dose levels in the dog. Stimulation was applied by electrodes implanted on the vagus nerve or on nerves near the caudal sympathetic ganglion. The changes in heart-rate, which occurred when stimulations were applied, were attenuated as the level of curarisation was increased. The dose level at which heart-rate changes ceased to occur was the same level at which a previously operantly conditioned heart-rate response, together with EMG activity, also ceased to be evident. The experiment was limited in that only two dogs were used. However, the results of

this study by Black would suggest that, in the dog, d-tubocurarine has a blocking action on autonomic ganglia at dose levels which are sufficient to suppress EMG activity. A comparison of these results with those obtained for the rat again emphasises the dangers in generalisation of the effects of curariform drugs from one species to another.

One interesting point which arose from Black's experiment was that an increase in heart-rate occurred when the vagus was stimulated at high levels of curarisation. Since the vagus is a mixed nerve, Black suggested that the dominant parasympathetic effects were more susceptible to blocking by d-tubocurarine than sympathetic components. Langley (1918), working on cats, had proposed a similar order of effect on ganglionic blockade by curare. However, Waser (1959) concluded from stimulation studies on the sympathetic and parasympathetic nerves of the cat, that a greater dose level of d-tubocurarine was required to block parasympathetic nerves. This conflict in evidence precludes the conclusion that, since parasympathetic ganglia are not blocked by d-tubocurarine at the dose levels used in these studies of operant conditioning of heart-rate changes in the rat, then sympathetic ganglia are also unaffected. Although a blocking effect was not expected in sympathetic ganglia because the previous study (Chapter 2) had shown, successfully, operant conditioning of heart-rate increases, it is possible that those increases in rate were not produced by increases in activity of cardiac sympathetic nerves. However, evidence presented in the following chapter indicated that d-tubocurarine chloride did not produce a block in sympathetic ganglia at dose levels used in these experiments.

There is one further danger in the use of high dose levels of curariform drugs which is related to their blocking action by

competition with acetylcholine. In order to satisfy a theoretical requirement of operant conditioning, there must be some dependable afferent consequence of the response which is to be conditioned. There is a suggestion that d-tubocurarine may block the afferent nerves which convey this information. Although there is considerable evidence for afferent feedback systems for cardiovascular responses (Rushmer, 1961), they have received little experimental investigation. The exception is the feedback system from the carotid bodies. Eyzaguirre and Zapata (1968) have shown that the transmitter substance within the carotid bodies is acetylcholine and that its activity is readily blocked by d-tubocurarine. These effects were demonstrated on isolated preparations of the carotid bodies of the cat and, consequently, generalisation of the result to the intact animal is undesirable. The convincing demonstrations of operant conditioning of heart-rate changes in the curarised animal, which have been presented in previous chapters, would indicate that afferent feedback of heart-rate changes was not blocked.

The implication of the experiments which have been cited, is to stress that, if a meaningful interpretation of the results of experiments on operant conditioning of cardiovascular changes is to be made, then it is necessary to investigate fully the blocking effects of the drug used for any particular set of experimental conditions.

CHAPTER FIVE

OPERANT CONDITIONING OF HEART-RATE CHANGES IN THE IMMUNOSYMPATHECTOMISED, CURARISED RAT

The results of the experiment presented in Chapter Two indicated that it was easier to operantly condition heart-rate increases rather than decreases, when shock was used as a reinforcer. Early evidence in the physiological literature (e.g. Samaan, 1935; Brouha, Cannon and Dill, 1936) would suggest that cardiac acceleration is primarily produced by inhibition of vagal activity, the role of the sympathetic cardiac nerves being to provide tonus for the system. Adolph (1967) has described such heart-rate control as being "tonically restrained" and points out that it occurs in the larger mammals, such as dogs and humans, from which most of the early physiological evidence was accumulated. However, Adolph has demonstrated that the postnatal heart-rate of the rat is 'tonically prodded' and that the mechanisms for heart-rate control are sympathetically dominant. Increases in heart-rate in the rat are, therefore, primarily produced by increases in activity of the sympathetic cardio-accelerator nerves. In accordance with these suggestions, DiCara (1970) evidently believes that the increases in heart-rate produced in his studies on operant conditioning of heart-rate changes in the curarised rat, are mediated through the sympathetic cardio-accelerator nerves.

The evidence presented in the previous chapter indicated that d-tubocurarine may block sympathetic ganglia at dose levels sufficient to suppress EMG activity. If this were the case, then the increases in heart-rate produced in studies of operant conditioning of heart-rate changes in the curarised rat may not be under the direct influence of cardio-accelerator nerves but under the control of some other mechanism, possibly of neuroendocrine origin. An alternative explanation could be that the results of the experiment were produced

by the tendency of the experimental schedule to increase the baseline heart-rate over training. Consequently, the increase in heart-rate response, which appears to have been operantly conditioned, was an artifact of this baseline shift and, the differential effect between the group shaped for heart-rate increases and the group shaped for decreases was produced totally by success in shaping decreases in response through vagal parasympathetic activity.

To determine whether the dose levels of d-tubocurarine chloride used in the experiments presented in this thesis did produce a block of the activity of the sympathetic cardio-accelerator nerves, an attempt was made to condition, by operant means, increases of heart-rate in sympathectomised rats. A technique called immunosympathectomy was used, which involves an immunological action to a specific protein, and which has several advantages over other methods of producing a functional sympathectomy (Van-Toller, 1970).

IMMUNOSYMPATHECTOMY

Levi-Montalcini has published a series of studies (Levi-Montalcini and Booker, 1960a; Levi-Montalcini, 1962; Levi-Montalcini, 1964; Levi-Montalcini, Shenkein, Bucker, Crain, Benitez and Watter, 1964; Levi-Montalcini and Angeletti, 1966) describing a Nerve Growth Factor (NGF) which, when isolated and purified, produced hypertrophic growth in the thoracolumbar sympathetic ganglia in any mammal that was injected with it. The greatest effects produced by the NGF are seen in neonatal animals when tissue development and hyperplasia are still evident. Following the discovery that the activity in NGF was due to a protein, NGF antiserum was produced in donor animals (Cohen, 1960). This NGF antiserum contains gamma globulin which has specific activity directed against the sympathetic nervous system of any mammal injected with the serum of the donor animal (Levi-Montalcini and Cohen, 1960;

Vogt, 1964; Zaimis, 1967). The hypotrophic growth produced by the NGF antiserum is permanent (Levi-Montalcini and Booker, 1960b) and if the NGF antiserum is injected into neonatal animals, the adult later shows a poorly developed sympathetic nervous system.

Early evidence for the success of this technique in producing sympathectomised animals was based largely on histological evidence. Vogt (1964) and Zaimis, Berk and Callingham (1965) have shown that the degree of hypotrophy was greatest in the thoracic and cervical ganglia, but considerably less in the coeliac and mesenteric ganglia. Detailed discussion of the effects of NGF antiserum, both in vivo and vitro, is given in articles by Sabatini, Pellegrino, Iraldi and de Robertis (1965) and Zaimis (1967). The evidence from anatomical studies would suggest that NGF antiserum has a strong hypotrophic effect on those nerves which affect cardiac responses, and there is supporting evidence that immunosympathectomy does produce a functional effect on such responses. Brody (1964) investigated the effects of several types of sympathetic stimulation on the cardiovascular activity of acute preparations of immunosympathectomised rats. He found that the vasomotor functions of the sympathetic nervous system were abolished and that no such response could be obtained on electrical or chemical sympathetic stimulation. However, he also pointed out that the baseline heart-rate levels of intact immunosympathectomised rats were little different from the resting levels of normal animals. Wenzel, Carson and Chase (1966) have also shown that the resting heart-rates of immunosympathectomised mice were similar to those of control animals. However, significantly smaller increases in heart-rate were found in the immunosympathectomised animals in aversive learning situations. Carson (1970) has collected recordings of the telemetered heart-rate responses of immunosympathectomised

and control mice during behavioural tests in the open-field and an automated shock runway, as well as during rest periods in the home cage. The consistent findings of Carson's study was that the immunosympathectomised mice had lower baseline heart-rates than control animals. It was also shown that, although the level of tachycardia response to shock stimulation was reached by immunosympathectomised mice as quickly as controls, it was maintained at such levels for much briefer periods.

Although there are some discrepancies in the results of these experiments, particularly with reference to baseline response rates, they all indicate a disturbance in cardiac function in immunosympathectomised animals. This conclusion is further supported by the study of Willard and Fuller (1969), who demonstrated that the hypertension produced in normal rats by an injection of the drug l-triiodothyronine, does not occur when a similar injection is given to immunosympathectomised rats. In their study, evidence was presented to show a markedly reduced uptake of noradrenaline to the heart of immunosympathectomised rats and, since it is known that an intact nervous system is necessary for the binding or release of exogenous noradrenaline (Bhagat, 1967; Iverson, 1967), this result supports the evidence for sympathetic reduction to the heart in these animals. Other studies (Levi-Montalcini and Angeletti, 1962; Visscher, Lee and Azuma, 1965) have shown similar changes of catecholamine levels in organs innervated by the paravertebral ganglia of the sympathetic system of immunosympathectomised animals. These studies further confirm that a functional sympathectomy is produced by immunosympathectomy.

EXPERIMENT

METHOD

Subjects: Immunosympathectomised and Control Rats.

One hooded female multiparous rat, after mating with a male, was isolated in a breeding box. Towards the termination of pregnancy the

female was checked at intervals of twelve hours, or less, for the presence of the litter and, as soon as possible after the birth, the female was gently ushered out of the nest box, which was then sealed off temporarily with a metal plate to prevent the female from returning during the initial sorting and injection of the litter.

The twelve neonatal rats from the litter were weighed and allocated to one of two groups consisting of neonates of approximately equal body weights. Each group was sorted into a separate paper lined metal enclosure, which was placed on a surgical warming table to reduce any detrimental hypothermic effects whilst handling the neonates (Schaefer, 1963; Hutchings, 1963). One group, the control group, was tail-clipped for identification purposes.

Injection Procedure

In 1967 the Wellcome Research Laboratories succeeded in producing a high titre NGF antiserum (9,600 anti-units/ml) from a horse, and after preliminary tests, small quantities of this freeze dried antiserum were made available for use in the Department of Psychology, University of Durham. A 10ml phial of the equine NGF antiserum was dissolved in 5ml of sterile distilled water which gave a double strength NGF antiserum of 19,200 anti-units/ml. Injections to the control group of neonates (NHS) were normal horse serum, in order to eliminate for possible effects due solely to gamma globulin. During the injection programme both the NGF antiserum and the normal horse serum were stored at 3-4^{o}C.

Before injection, the sera were allowed to warm up to room temperature. The appropriate volume of sera, 0.6ml, on each day was drawn, under sterile conditions, into sterilised 1ml TB syringes mounted on No.1 Record hypodermic needles.

After again confining the female to the outer part of the breeding box, the neonates were sorted into the tail-clipped and

unclipped groups by placing them into the warm, insulated metal enclosure. Each neonate was injected subcutaneously into the nape of the neck and along the midline of the back with 0.1ml of the appropriate serum. Van-Toller (1970) had shown that if the tip of the needle was gently directed away from the midline of the back into the fold of skin in front of the hind limb, leakage of the serum after the withdrawal of the needle was prevented. Injections of the same volume of the appropriate serum were given to each neonate using the same procedure, at twenty-four hour intervals for eleven days post partum. After each injection the neonate was replaced into the nest box and not disturbed by the experimenter until the next injection period. On the twelfth day post partum, a hole was punched in the left ear of the IS animals and a hole in the right ear of the NHS animals to allow for a permanent and rapid identification of the treatment of any animal. An animal technician, who was naive of the treatment each animal received, noted eyelid droop in the six IS animals. This effect, known as ptosis sympatheticus, has been used previously by Zaimis (1967) to distinguish IS and normal rats.

Procedure for operant conditioning of heart-rate changes

The basic apparatus and procedure for shaping heart-rate changes has been described in Chapter Two. However, subsequent to the analysis of the results of that experiment, the procedure was modified in three fundamental respects.

(i) Artificial ventilation was maintained by a commercial respirator (E. & M. Instrument Co.), with an active expiration stroke because of the advantages of this method, which were fully discussed in the previous chapter. The animals were ventilated at 70 cycles per minute, with a peak pressure of 12cm of water and a 1:1 inspiration to expiration ratio.

(ii) No attempt was to be made to demonstrate discrimination learning of heart-rate changes between reinforced and non-reinforced stimulus conditions. Consequently, non-reinforced, CS- trials in the schedule were replaced with further blank trials, in which heart-rate was again counted in the absence of any stimulus. The signal for operant, CS+ trials was the 60 watt light for all the animals. Each block of thirty training trials consisted, therefore, of 10 CS+ trials and 20 blank trials.

(iii) Since considerable difficulty had been encountered in shaping decreases in heart-rate, the percentage shifts in criterion level for animals in the decrease group were lowered. Moreover, remedial changes in criterion were made if more than 20 shock pulses were received by an animal on any two consecutive CS+ trials.

These three modifications in procedure were also incorporated into every subsequent experiment which is presented in this thesis.

For this experiment on operant conditioning of heart-rate changes of IS rats, four of the six animals in the IS group were operantly shaped for heart-rate increases and the remaining two were shaped for decreases. Similar numbers of NHS rats were assigned to increase and decrease groups. The number of animals used in the experiment was limited because of the limited availability and the high expense of the sera.

Physiological Measurements

Four weeks after operant training the animals were weighed and then killed over ether and the pairs of cervical and thoracic sympathetic ganglia, together with the adrenals, were dissected out, cleaned of connective tissue and fat, and then weighed.

RESULTS

There were no apparent differences in the response rate to CS+ and blank trials, and consequently the results of the training schedule are analysed in eight blocks of operant, CS+ trials. Figure 5.1 presents the group mean

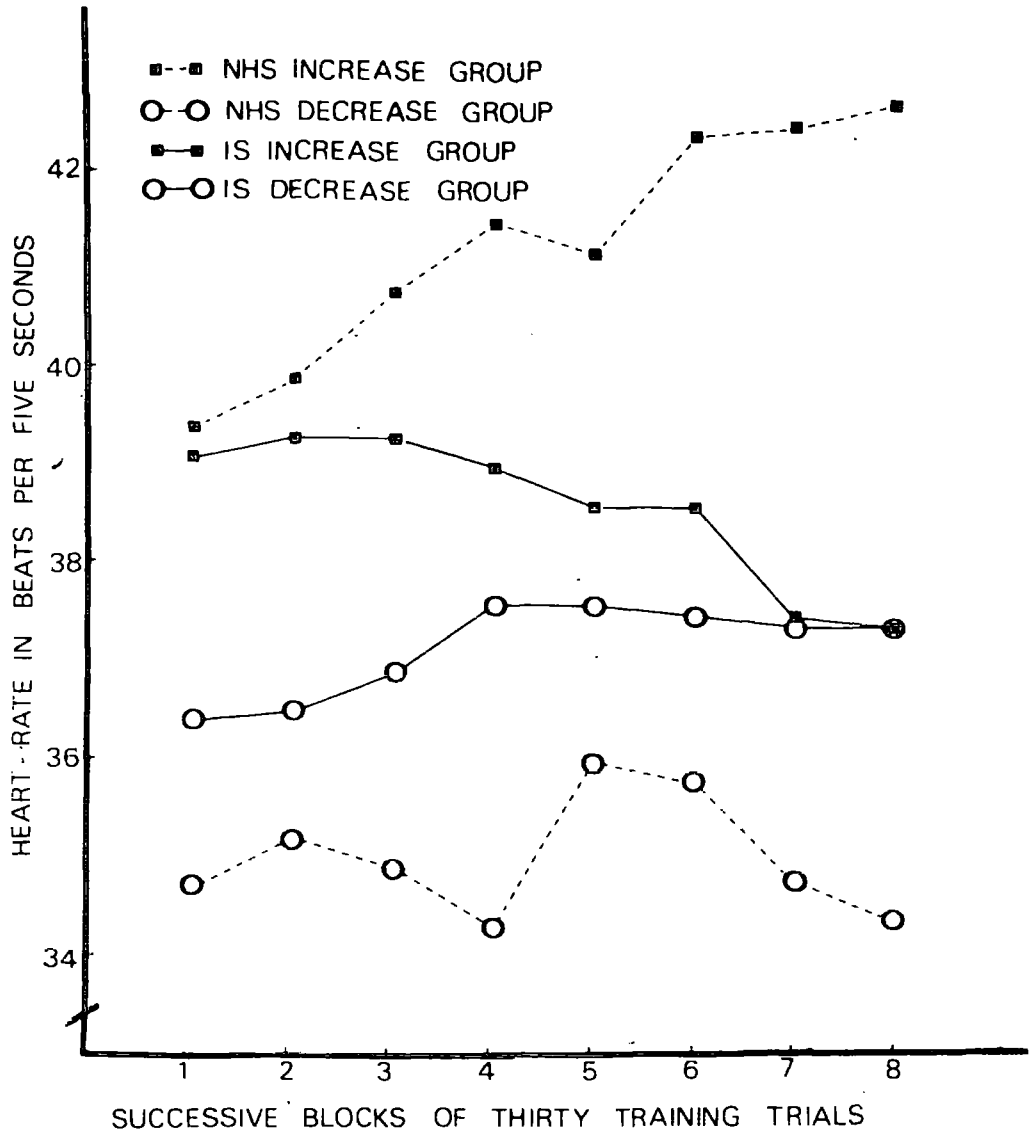

FIGURE 5.1 The mean changes in heart-rate, over successive blocks of training trials, of groups of immunosympathectomised (IS) and control (NHS) curarised rats shaped for either increases or decreases in rate. Control animals shaped for increases in rate show the appropriate change over training; increases in heart-rate, which are not evident in the immunosympathectomised animals shaped for that response.

changes in heart-rate over the eight blocks of training trials for both groups of IS and NHS animals. There is a clear discrepancy between the NHS increase group and the NHS decrease group, whilst little difference between comparative groups of IS animals. Moreover, the increase group of NHS animals shows the predicted increases in heart-rate response which are not evident in IS animals shaped for heart-rate increases. The absolute percentage change in heart-rate over the training period is presented for each animal in Table 5.A. A test for the difference in response between the NHS and IS increase groups gave a value of $t = 3.13$, 6df; $p > .05 < .02$. This difference would not appear to be solely produced by a general tendency for the NHS animals to increase their heart-rate under the experimental conditions because such a tendency was not evident for the two NHS animals shaped for decreases in rate. The mean increase in heart-rate response for NHS animals in the increase group was from 473 beats per minute to 512 beats per minute. This increase in rate is less than would have been expected from the results of the previous experiment which was presented in Chapter Two. Two factors, however, do not favour increase in heart-rate levels in this experiment. Firstly, the method of artificial ventilation used in this experiment tends to produce a slight decrease in the baseline heart-rate, as indicated in the previous chapter. Secondly, in accordance with the Law of Initial Values, the higher initial baseline rates of the IS and NHS increase groups do not favour further increases in the heart-rate responses of animals in those groups.

The mean number of shocks received by each animal per block of training trials is also presented in Table 5.A. A t-test for the difference between the IS and NHS increase groups for this parameter of shock frequency gave a value of $t = 2.49$, 6df; $p < .05$. Animals

TABLE 5.A The percentage change in heart-rate over training attained by each immunosympathectomised and control animal, together with the frequency and probability of receiving shocks during training by that animal.

Column headings:

S = Animal number.

A = Percentage change in heart-rate over training attained by each animal. Negative numbers indicate a decrease in rate.

B = The mean number of shocks received per block of ten operant trials throughout training.

C = The mean number of trials per block of ten operant trials on which a shock was received during training.

NHS				IS			
increase group				increase group			
S	A	B	C	S	A	B	C
1	16·3	12·1	3·9	1	-3·4	20·8	4·1
2	1·6	20	2·7	2	-0·3	20·3	4·3
3	8·1	16·6	3·5	3	-12·8	24	3·4
4	8·6	17·4	3·5	4	-1	19·9	4·2
decrease group				decrease group			
5	-2·7	15·6	4·3	5	0·6	15·4	3
6	1·2	19·3	4·1	6	-3	21	3·8

in the IS increase group tended to receive only slightly more shocks than animals in the NHS increase group. This is evident from the data presented in Figure 5.2 as a plot of the percentage change in the heart-rate response of each animal over the training period, and the mean number of shocks received per block of trials by that animal. There appears to be little difference between the increase group of IS and NHS animals in the mean number of trials per block of trials on which a shock was received. This is evident from Figure 5.3 which presents a plot of this parameter of shock probability for each animal against the percentage change of the heart-rate response over the training schedule for that animal. The absolute values for each animal of this parameter are given in Table 5.A and a t-test confirms the absence of any significant difference between the IS and NHS increase groups ($t = 1.85$, 6df; $p > .2, < .1$). The data indicates that the differences in heart-rate responses between the increase groups of the NHS and IS animals were not produced by differences in the shock parameters.

The body weight of each animal at the time of dissection, together with the weight of the two adrenal glands and the combined weight of the four sympathetic ganglia is presented in Table 5.B. It is clear that the weights of the ganglia of IS rats were considerably lower than those of the NHS controls. However, in accord with other studies on the effects of immunosympathectomy, there is no differential effect on the weights of the adrenal glands.

DISCUSSION

It is clear from the results that a reduction in function of the sympathetic system, produced by the technique of immunosympathectomy, has a profound effect on the success of producing increases in heart-rate by means of operant conditioning principles. It can be concluded

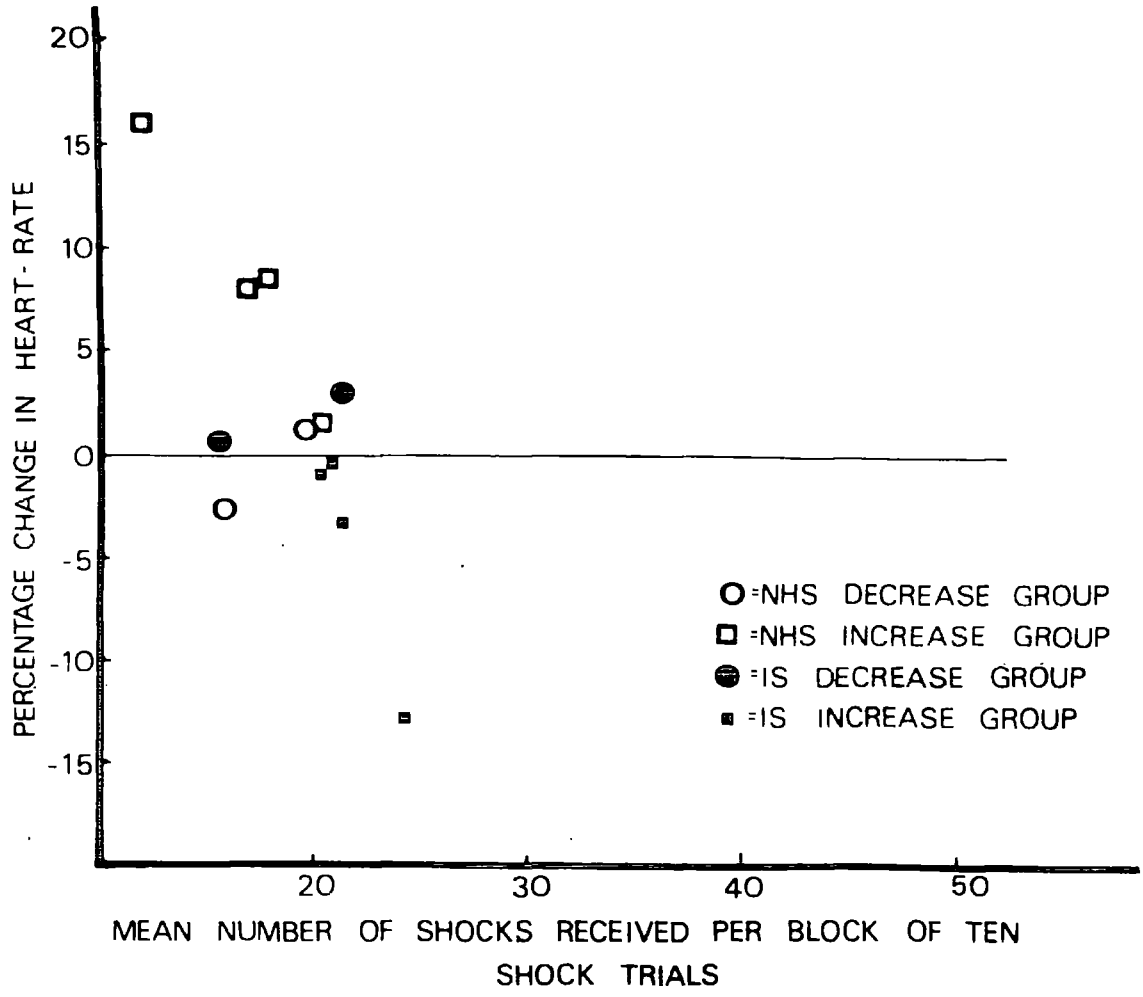

FIGURE 5.2 A plot of the mean number of shocks received per block of ten operant training trials and the percentage change in heart-rate over the training period for each immunosympathectomised and control rat. Although the plot suggests that the IS rats shaped for increases in rate received fewer shocks than control NHS rats shaped for similar responses, the differences between these groups is slight and, statistically, is non-significant.

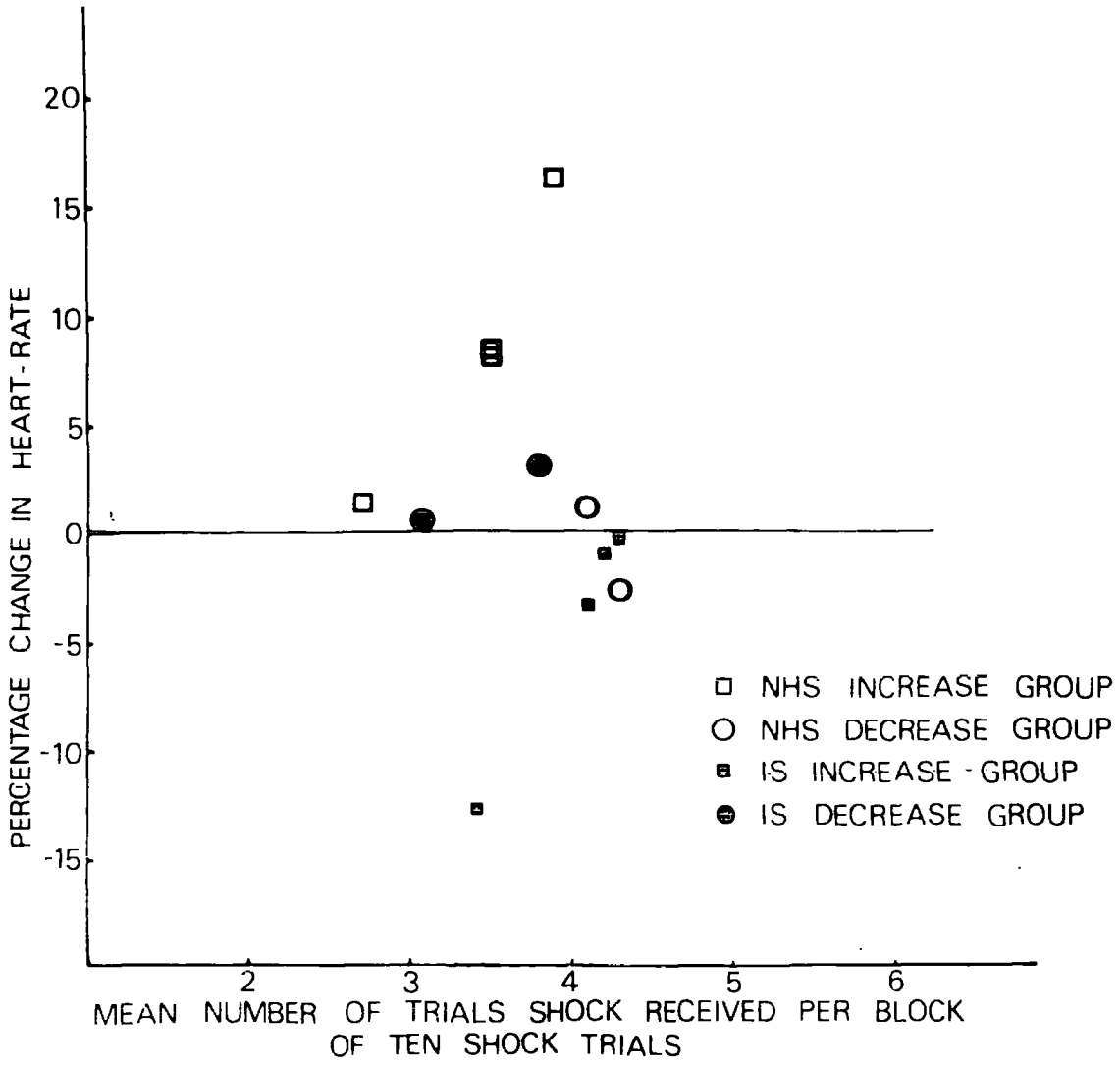

FIGURE 5.3 A plot of the mean number of trials per block of ten operant trials on which a shock was received by each immunosympathectomised and control animal, and the percentage change in heart-rate over the training period by that animal. There is apparent an overlap in the probability of receiving a shock on any one trial between IS and NHS rats shaped for increases in heart-rate.

TABLE 5.B The body weight and the tissue weight of adrenal glands and sympathetic ganglia dissected out from each immunosympathectomised and control rat. The adrenal weight is the combined weight of the two glands. The weight of the ganglia is the combined weight of the two cervical and two thoracic sympathetic ganglia.

	Body Weight in gm.	Combined Adrenal Weight in mgm.	Combined Adrenal Weight in mgm.
N H S	372	1.372	6.640
	345	1.655	6.174
	236	0.990	6.530
	224	1.146	7.400
	288	1.421	6.230
	210	1.179	5.890
I S	335	0.667	6.500
	342	0.237	5.940
	295	0.554	6.740
	219	0.264	5.950
	221	0.480	6.536
	213	0.289	6.721

from this that d-tubocurarine does not produce a block in sympathetic cardio-accelerator nerves of the rat at the dose levels used in this series of experiments, which completely suppress EMG activity. This conclusion is again in direct conflict with the conclusions of Black (1967) on the effects of d-tubocurarine on the sympathetic cardiac nerves in the dog, and emphasises the need for caution in the use of curariform drugs in studies of autonomic conditioning.

The absence of success in shaping heart-rate changes in IS animals is not a function of a decrease in the variability of cardiac rate, since variations and oscillations of rate, unrelated to sinus arrhythmia, were clearly seen in the cardiotachometer records obtained from the animals during operant training. Those heart-rate responses may be due to changes in vagal tone. Carson (1970) has observed similar variations and oscillations in heart-rate of immunosympathectomised mice in both the home cage, and a variety of behavioural situations. However, her interpretation of these results is that they are possibly a consequence of alterations in the feedback mechanisms which control heart-rate. If this is a correct interpretation, then difficulty in shaping heart-rate changes in IS animals may be non too surprising, since a pre-requisite for operant learning is rapid feedback of dependable response associated cues.

The cardiotachometer recordings also show that the effect of shock is to produce tachycardia in the IS animals as well as in the NHS controls. Such increases could occur through either a reduction in vagal tone or, alternatively, by other possible sources of catecholamines which may compensate for the effects of sympathectomy. There is experimental evidence for such compensatory effects. Trendelenburg (1966), reviewing the evidence for denervation supersensitivity in the autonomic nervous system, has reported compensatory changes which overcome any imposed reduction on the

influence of that portion of the nervous system. Two studies have indicated possible compensatory mechanisms in immunosympathectomised animals. Carpi and Oliverio (1964) have shown that, although the levels of urinary noradrenaline from IS rats were below the sensitivity levels of their assay technique, an injection of amphetamine produced large increases in the noradrenaline levels of IS rats compared to control animals. The authors concluded that the large increases in the IS rats indicated a noradrenergic system which entered into action following the reduction of the sympathetic system. Schönbaum, Johnson and Sellars (1966) showed that IS rats have considerable noradrenergic reserves when subjected to cold stress but they noted that, if the stress was made more severe by clipping the fur, the IS animals were unable to maintain the functioning of their hypotrophic sympathetic system.

The evidence for compensatory sources of noradrenaline in IS animals, together with the results of Carson (1970) and Schönbaum et al (1966), would support a hypothesis that, although IS animals are able to increase their cardiac rates, they are unable to maintain them at high levels. The hypothesis would also be supported by this study on operant conditioning of heart-rate changes in IS rats, since no success was obtained in shaping increases in the heart-rate response. This concept was first postulated by Ramey and Goldstein (1957) to account for the effects of sympathectomy and adrenalectomy in a variety of stressful situations and the effects were seen as a reduction in 'the adaptive range of response'.

CHAPTER SIX

CLASSICAL CONDITIONED AND UNCONDITIONED HEART-RATE COMPONENTS IN STUDIES OF OPERANT CONDITIONING OF HEART-RATE CHANGES

In Chapter Two, it was suggested that the results of this experiment on operant conditioning of heart-rate changes may have been influenced by both the effects of d-tubocurarine on autonomic ganglia and also the effects of differences in shock parameters between animals shaped for heart-rate increases and animals shaped for decreases. Although it was suggested that these differences could not completely explain the heart-rate changes obtained between the two groups, it will be attempted to present further evidence for this conclusion.

As stated previously, the very nature of the reinforcer used (i.e. shock), with its unconditioned effect on heart-rate, makes it improbable that the numbers of shocks received by both the increase and decrease groups would be similar. The use of a time-out period after each reinforcement (Black, 1967; Hothersall and Brener, 1969) whilst countering the effects of the unconditioned cardiac response, did not completely solve the problem, since the direction of the classical conditioned heart-rate component would undoubtedly influence the probability of avoiding the shock on any one trial. The classical conditioned component of the heart-rate response not only affects the probability of receiving a shock and, therefore, the unconditioned cardiac response, but also the varying probability of a shock being received by an animal itself affects the direction and degree of heart-rate changes (Fitzgerald, Vardaris and Teyler, 1966; Caul and Miller, 1968; Miller and Caul, 1969).

Two approaches were taken in order to confirm that, using the bidirectional design, it is possible to demonstrate that operant manipulations effect changes in heart-rate in a manner which cannot be explained solely in terms of the unconditioned and classical conditioned

cardiac components to reinforcement presentations. One of these approaches was indirect, and the results have implications beyond the attempts to control for shock parameters. The second approach was by a direct attempt at determining the effects of the presentations of shock on the heart-rate response in the absence of an operant schedule.

1. THE INDIRECT APPROACH: AN ATTEMPT AT OPERANT CONDITIONING OF HEART-RATE CHANGES IN THE FUNCTIONALLY DECORTICATE CURARISED RAT.

Cortical Spreading Depression.

The technique applied to the problem outlined above was that of cortical spreading depression. The general principle of the technique was first elaborated by Leão (1944, 1947) who showed that strong electrical stimulation, certain chemical substances, mechanical deformation, or prolonged exposure of the cortical surface of an animal produced a slow high amplitude ~~direct current~~ DC shift in potential of the cortex. These ~~direct current~~ DC changes spread gradually in all directions from the point of stimulation with a velocity of 2.6 mm per second. The essential point is that, during and immediately following the passage of the direct current wave, all signs of neural activity are inhibited (as indicated by changes in electro-encephalograph recordings). The spread of this depression is confined to the cerebral cortex. However, other brain areas are affected; Liberson and Cadilhac (1953) and Liberson and Akert (1953) have shown alterations in the electrical activity of the hippocampal formation by cortical spreading depression; Weiss and Fifkova (1961) demonstrated effects in the thalamus, and Bureš, Burešova, Fifkova, Olds, Olds and Travis (1961) showed changes in the bulbopontine and mesencephalic reticular formation. Fortunately, the effects in these subcortical regions are not direct, but are a consequence of the loss of cortical influence on these areas.

Accounts of the mechanism of action and more detailed effects of cortical spreading depression can be found in a review by Marshall (1959) and in the texts of Ochs (1965) and Grossman (1967).

The Effects of Spreading Depression on Learning and Memory.

Several studies have demonstrated the effects of spreading depression on learning: Bureš, Burešova and Zahorova (1958) and Bureš and Burešova (1960) have shown that animals under bilateral spreading depression exhibit severe impairment in reflexive and learned activities involved with food getting, and avoidance of a noxious stimulus. These studies were confounded by the experiments of Shima (1964), Mogenson (1965), and Steele Russell, Plotkin and Kleinman (1968) which indicated that spreading depression produced impairment in motor functions, as reflected in postural and locomotor change and by deficits in escape behaviour from painful electric shocks. Tapp (1958) suggested that rats were able to retain emotional components of a habit under spreading depression but were unable to integrate the motor behaviour necessary for the correct avoidance response. This suggestion was followed by the very important experiment of Mogenson and Peterson (1966) which demonstrated that spreading depression has little or no influence on involuntary or environmentally triggered behaviour, as indicated by conditioned and unconditioned cardiac responses. It is of interest that Bloch and Lagarrigue (1968) have shown a dissociation of cardiac responses and somatic responses in neodecorticate cats, with the cardiac responses being unaffected. This would seem to confirm the suggestion of a functional similarity between decortication and spreading depression. Carlson (1967) has implied that the storage of emotional and cue components of passive and active avoidance learning situations can be subcortical, whilst complex motor components require an intact cortex. Steele Russell (1966)

has presented further studies which also indicate that only the more complex operant behaviour requires the functional integrity of the cerebral cortex. He concluded, from the experimental evidence available, that the deficits in operant behaviour in functionally decorticate animals were due to a deficit in operant learning and the complexity of the tasks, rather than from any motor impairment.

Operant Conditioning of Heart-Rate Changes in the Curarised Rat Under Conditions of Cortical Spreading Depression.

From the evidence on the effects of functional decortication on learning and memory, it would be expected that attempts to operantly condition changes in heart-rate responses in the curarised rat would prove unsuccessful if the cerebral cortices of that animal were depressed by use of the technique suggested by Leão (1944). Moreover, a detrimental effect on such learning would be unlikely to be a consequence of response impairment, since the physiological mechanisms for the control of heart-rate changes are primarily located in the lower brainstem.

If this hypothesis for a deficit in operant conditioning of heart-rate changes in the functionally decorticate rat were to prove correct, it would have important implications for other studies presented in this thesis on operant conditioning of heart-rate changes in which a bilateral design is used, and in which unequal numbers of the primary reinforcer, shock, are received by the groups of animals shaped for increases or decreases in rate. Since the unconditioned and the classical conditioned components of heart-rate changes are unaffected by spreading depression, then the probabilities of escaping and avoiding shock by the increase and decrease groups should be unaltered. If, in the experiment presented in Chapter Two, the differences between the increase and decrease groups in the

heart-rates obtained were merely due to differences in shock parameters, and not to the operant training schedule, then similar differences in rate could be expected to be obtained in a replication of that experiment in which the curarised rats were functionally decorticated by spreading depression. An absence of a differential effect in the heart-rate response between the groups, together with an expected difference in shock parameters, would support the previous suggestion for operant manipulations of heart-rate changes.

EXPERIMENT

METHOD

Subjects

Sixteen neonatal hooded rats were obtained from two litters of animals maintained in the Department of Psychology, University of Durham. Each of these rats was isolated 28 days after birth and used later in the experiment when it weighed between 250-300 gm. These rats were larger than those used in previous experiments in order to facilitate the operative procedure.

Operative Procedure

A chronic preparation was required to administer potassium chloride in order to produce the cortical spreading depression. The technique used was similar to that suggested by Schneider and Behar (1964) although sufficient modifications were involved to warrant a detailed description.

The operative procedure was carried out under Nembutal anaesthesia (40mg/Kgm body weight, injected intraperitoneally). A midline incision was made along the scalp and, after scraping the periosteum from the surface of the skull, circular fenestras, 0.4 mm diameter, were

trephined in each side of the skull to expose the brain surface of each hemisphere. Care was taken not to bruise or damage the dura. Each fenestra was situated midway in the parietal bone, about 4 mm caudal to the coronal suture and with the closest edge 2 mm from the mid-sagittal plane. A perspex cannula 1 cm diameter was located to the skull over the fenestras by stainless steel screws and fixed with dental cement. Finally, the skin was sutured to the assembly with stainless steel wire through small holes drilled in the flange around the top of the cannula. This procedure was found to be successful in keeping the assembly fixed to the skull. A diagram of the cannula fitted to the skull of the animal can be seen in Figure 6.1. At the end of the operative procedure, the cannula was plugged with a sterile pad soaked in isotonic saline and then capped with a plastic top. As a precaution against infection, the animal was given a subcutaneous injection of penicillin (6,000 units) immediately after the operation.

Animals were checked daily for infection under mild anaesthesia when, on each occasion, fresh sterile isotonic saline pads were inserted into the cannula.

Procedure for Producing Cortical Spreading Depression

Four days after the operative procedure, the rat was briefly anaesthetised with ether, the pads in the cannula removed and filter paper soaked in a 25 percent solution of potassium chloride was placed on the exposed surfaces of the dura. The cannula was then capped and the rat allowed to recover from the anaesthesia.

This procedure for producing spreading depression had been followed for six non-experimental rats. In these animals, electro-encephalograph (EEG) recordings were taken under mild ether anaesthesia

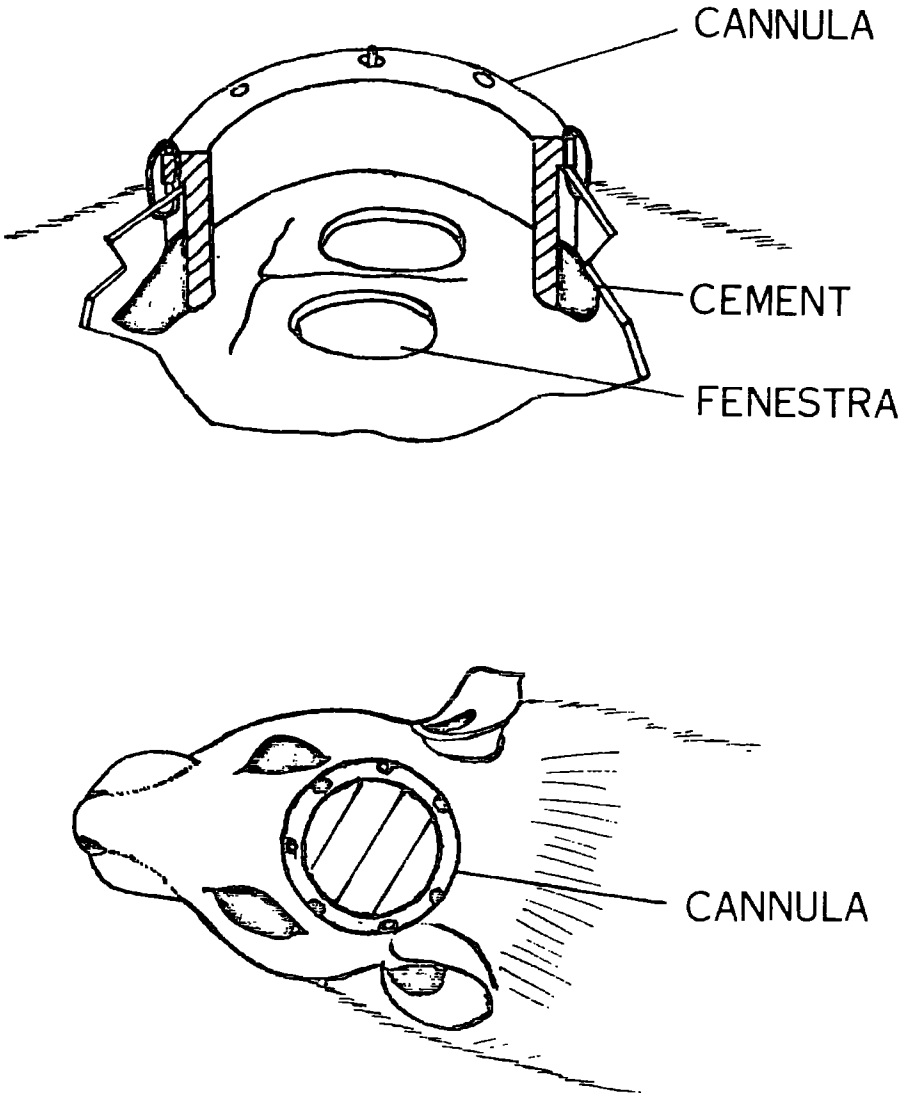

FIGURE 6.1 A cut-away diagram of the perspex cannula attached to the skull of the rat for purposes of spreading cortical depression by application of a solution of potassium chloride to the exposed dura.

both before and after the application of potassium chloride to the dura. The electrodes for recording the electrical activity of the brain were stainless steel needles connected to the skull. It was found that an almost complete elimination of neural activity, as measured by changes in the EEG, was obtained in every rat after the application of the potassium chloride solution (Figure 6.2). This suppression of electrical activity was found to last for periods of $2\frac{1}{2}$ hours. Although it was not possible to take EEG recordings from experimental rats during operant conditioning of heart-rate changes, the consistent effectiveness of the procedure for spreading depression in non-experimental animals, would firmly suggest that the technique was successful in producing a similar effect in the experimental animals during operant training.

Procedure for Operant Conditioning of Heart-Rate Changes in the Curarised Rat Under Spreading Depression.

The apparatus and procedure for operant conditioning of heart-rate changes in the curarised rat have been described previously (see Chapter Five). The procedure for producing cortical depression was initiated immediately prior to the initial injection of d-tubocurarine chloride. Eight rats were assigned to the group shaped for increases in heart-rate and eight to the group shaped for decreases in heart-rate. However, owing to failure in artificial ventilation, one animal in the decrease group died and, consequently, results were obtained for only the fifteen remaining animals.

RESULTS

As in the previous experiments, the training sessions were analysed in eight blocks of trials. Within each block of trials there were ten operant CS+ trials and twenty blank trials. No differences were observed in heart-rate response between operant CS+ and blank trials.

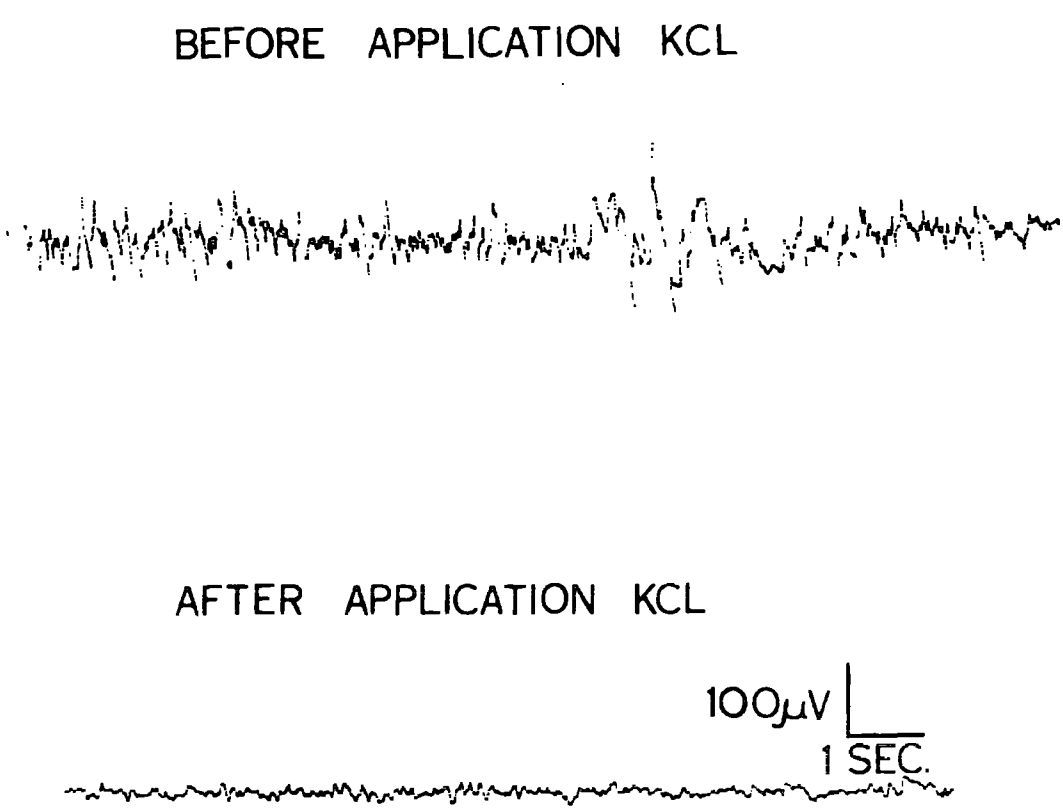

FIGURE 6.2 The EEG recorded from the skull of the mildly anaesthetised rat both before and fifteen minutes after topical application of a 25 per cent solution of potassium chloride to the exposed dura of both hemispheres of the brain. A reduction in gross activity is clearly seen after the application.

The group mean changes in heart-rate over the eight successive blocks of operant training trials, for animals shaped for heart-rate decreases, together with the changes for animals shaped for increases in heart-rate, are presented in Figure 6.3. It can be clearly seen that there is very little change in either group mean heart-rate response over the training period. The percentage change in heart-rate of each animal in both groups, over the training period is presented in Table 6.A. An analysis confirms the absence of any significant difference between the changes obtained in the heart-rate responses between the increase and decrease groups of rats ($t = 0.22$, 13df).

The mean number of shocks received per block of operant trials by each rat is also presented in Table 6.A. A statistical test for the difference between the increase and decrease groups of rats for this parameter of shock frequency gave a value $t = 3.20$, 13df; $p > .01 < .001$. This difference can be clearly seen in Figure 6.4 which presents a plot of the percentage change in heart-rate which was obtained for each rat and the mean number of shocks received per block of operant trials by that animal.

The mean number of trials per block of operant trials on which a shock was received is presented in Figure 6.5 for each rat as a plot against the percentage change in heart-rate of that animal over the training period. The apparent absence of a difference between the values of this parameter of shock probability for animals shaped for heart-rate increases and those shaped for decreases is confirmed by statistical analysis of this difference ($t = 0.36$, 13df). The absolute values of this paramter for each rat, in both groups, are presented in Table 6.A.

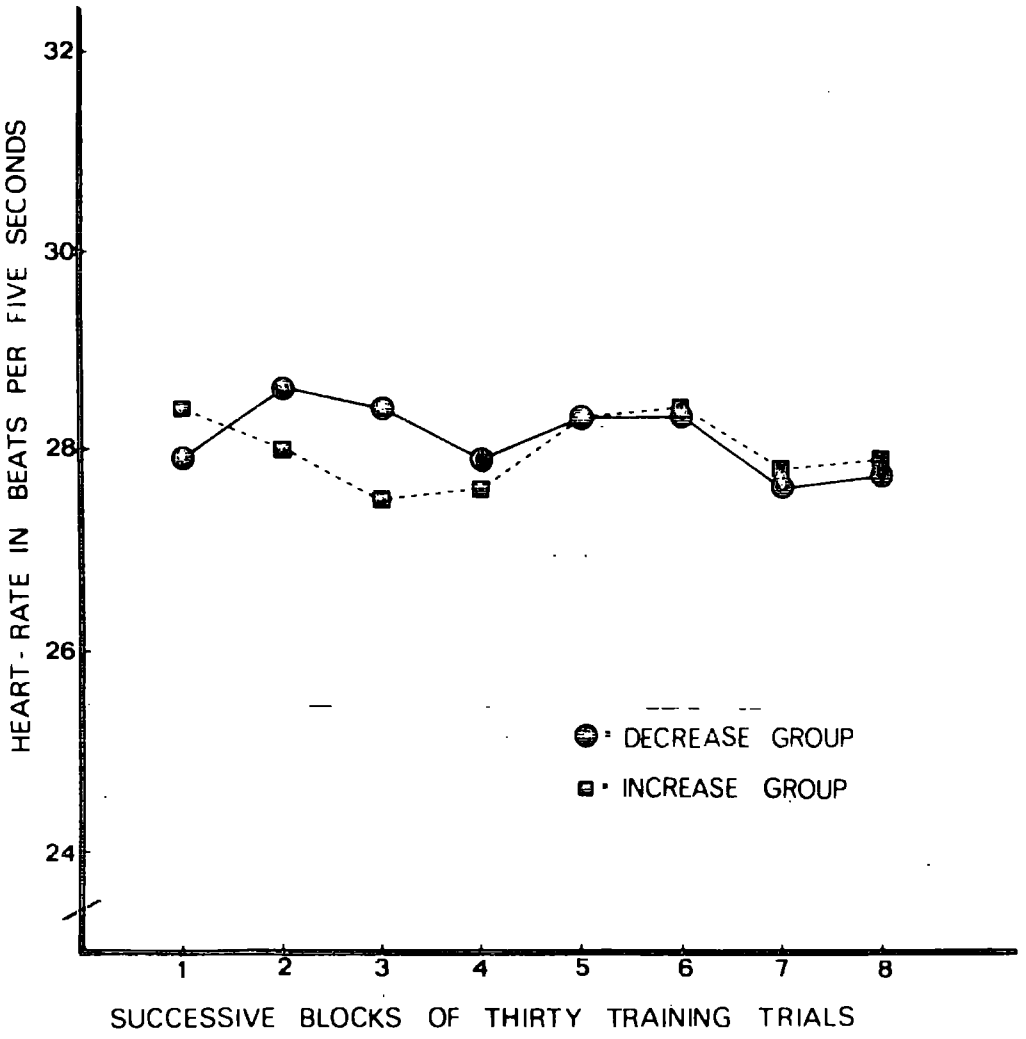

FIGURE 6.3 The mean heart-rate response of the groups of functionally decorticate, curarised rats shaped for either increases or decreases in rate both appear to show little change over the training trials despite operant contingencies in effect.

TABLE 6.A The percentage change in heart-rate over training attained by each functionally decorticate, curarised rat, together with the frequency and probability of receiving shocks during training by that animal.

Column headings:

A = Percentage change in heart-rate over training trials. Negative numbers indicate a decrease in rate.

B = The mean number of shocks received per block of ten operant trials throughout training.

C = The mean number of trials per block of ten operant trials on which a shock was received during training.

INCREASE GROUP				DECREASE GROUP			
SUBJECT	A	B	C	SUBJECT	A	B	C
1	2·1	23·9	5·3	1	4·3	18·4	5·2
2	11·5	12·6	2·5	2	-7·4	28·4	2·8
3	0	20·3	3·4	3	4·9	25·4	3·8
4	-5·5	10·5	3·6	4	-1·5	23·6	4·4
5	-1·7	18·4	4·2	5	0	23·5	4·5
6	-1·5	11·1	4·3	6	2	21·3	4·3
7	-6·9	14·3	4·3	7	-1·4	19	2·9
8	-1·6	15·6	5·7	8	NO DATA		

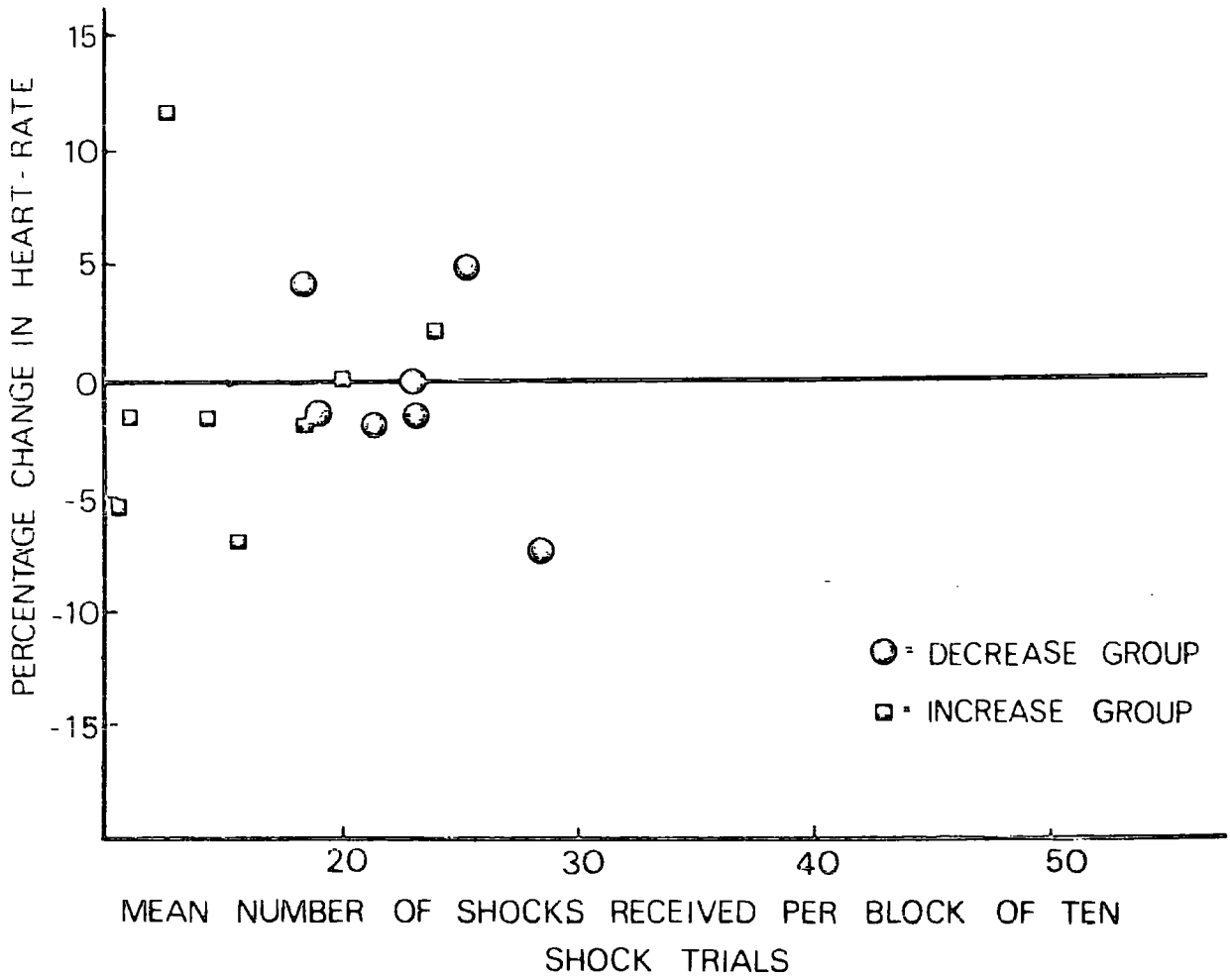

FIGURE 6.4 A plot of the mean number of shocks received per block of ten operant trials and the percentage change in heart-rate over the training for each functionally decorticate, curarised rat. The plot indicates that fewer shocks were received by animals shaped for heart-rate increases than by rats shaped for decreases.

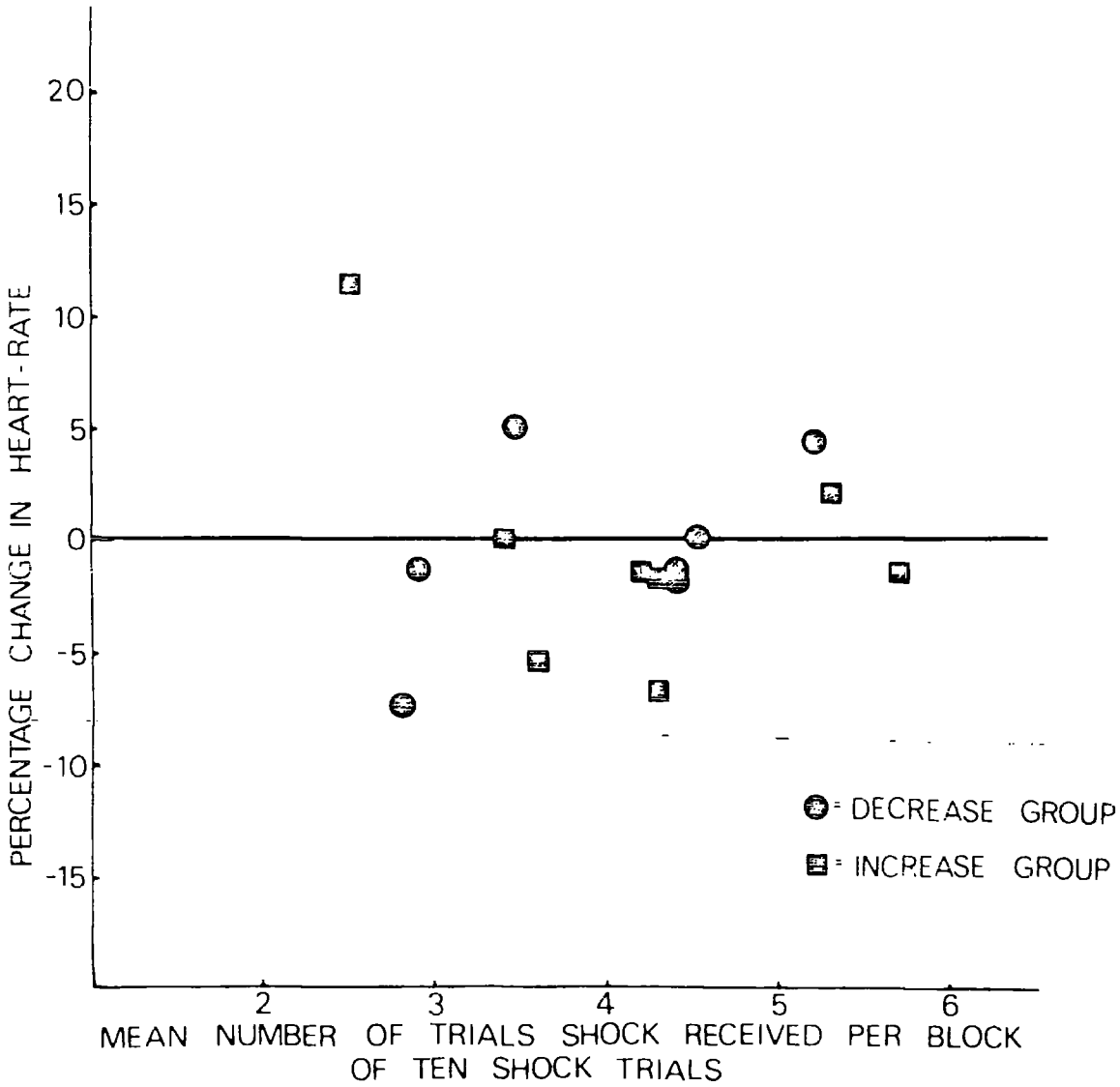

FIGURE 6.5 A plot of the mean number of trials per block of ten operant trials on which a shock was received by each functionally decorticate, curarised animal, and the percentage change in heart-rate over the training period. There appears to be considerable overlap in the probability of receiving a shock on any one trial between animals shaped for increases or decreases in heart-rate.

DISCUSSION

The results of this experiment clearly show that little change is produced in the heart-rate response of either group of cortical depressed, curarised rats, despite the operant schedules which were in effect. The absence of any difference in the heart-rate response between the increase and decrease groups is a contrast to the result obtained in the previously presented experiment (see Chapter Two) on operant conditioning of heart-rate changes in the curarised rat. The results seem, therefore, to confirm the hypothesis that operant conditioning of heart-rate changes does not occur in the absence of cerebral cortical functioning.

If the classical conditioned and unconditioned components of the heart-rate response were unaffected by spreading depression, as suggested by Mogenson and Peterson (1966) then it would be expected that the numbers of shocks received by animals shaped for increases in heart-rate would be different from the numbers received by animals shaped for decreases in rate. This is because the reported tachycardia to shock stimulation, which was again observed in the cardiotachometer recordings from animals in this experiment, would increase the probability of escaping from shock for animals in the increase group. Consequently, the numbers of shocks received by animals shaped for decreases in rate were expected, and confirmed, to be greater than for animals shaped for increases in rate.

The result of this experiment would, therefore, confirm that the differences in the heart-rate responses obtained in the previously reported experiment were not produced by differences in shock parameters between the two groups, but were a direct consequence of the operant schedule.

The findings of this experiment also provide additional support for Steele Russell's hypothesis of separate neuroanatomical loci for

operant and classical conditioning. However, it is still not conclusive that the importance of the cerebral cortex is for the operant learning, per se, rather than for its importance on motor behaviour. Although the primary mechanisms of heart-rate control are situated in the lower brainstem, it has not been ascertained whether the changes in heart-rate, produced by operant conditioning of such changes in the curarised rat, are produced by direct effects on those control mechanisms, or whether they are mediated by operant conditioning of central motor cortical activity. This problem of mediation of autonomic responses is clearly complex. However, DiCara and Miller (1968a) have cogently argued against mediation of operant heart-rate changes by motor cortical activity and the results of his experiments (as yet unpublished) which are attempting to measure the activity of the motor cortex during operant conditioning of cardiac responses, should prove fruitful, even though they are likely to be fraught with difficulties because of the close integration of cardiac and motor cortical control mechanisms (Rushmer and Smith, 1959; Obrist, Webb, Sutterer and Howard, 1970).

It could be tenuously argued, that the absence of a differential heart-rate response in this study of operant conditioning of heart-rate changes in the functionally decorticate, curarised rat was not a function of the cortical depression, but was a consequence of the side effects arising from the surgical procedure and/or the minor differences in the operant training procedure which were made after the first preliminary experiment. Both these possibilities would seem to be refuted by other experiments which will be presented in this thesis. In these studies successful operant conditioning of heart-rate changes is shown to occur with exactly the same operant training procedure used in this experiment (see Chapter Seven). Unfortunately, there is no completely appropriate control for the surgical and technical procedure for producing cortical

spreading depression. The specific operative procedure of trephining fenestras in the skull and exposure of the brain, may in itself be sufficient to produce cortical depression (Leão, 1944, 1947) and, consequently, this procedure, in the absence of the application of potassium chloride solution, would not necessarily leave animals with a functionally intact cortex. The results of the experiment presented in the following chapter would indicate that the general surgical procedure does not interfere with the success in demonstrating operant conditioning of heart-rate changes in the curarised rat.

2. THE DIRECT APPROACH: THE USE OF THE YOKED CONTROL IN STUDIES OF OPERANT CONDITIONING OF HEART-RATE CHANGES IN THE CURARISED RAT.

The more obvious control for the effects of variations in the presentations of shock to animals in studies of operant conditioning of heart-rate changes is to use yoked animals. Each experimental animal has a paired control which receives the same number and distribution of trials and light and shock presentations but, for the yoked control, these are independent of its own heart-rate. However, the use of the yoked control in studies of operant conditioning of autonomic responses has been strongly criticised (see Kimmel, 1967; Murray and Katkin, 1968; Crider, Schwartz and Shnidman, 1969; Katkin, Murray and Lachman, 1969). Their criticism is based on the evidence which suggests that the autonomic response is activated by the unconditioned stimulus to different levels, in different subjects. This effect produces acute problems for studies in which a positive reinforcer is used, and where operant conditioning of the response in a single direction is attempted. If, in such studies, the unconditioned stimulus is a response activator, then a better experimental subject will improve in learning at the expense of a poorer yoked control but, the better control will not improve if it is yoked with a poorer experimental subject. These problems would be reduced when a bidirectional design is used, provided that the number of reinforcement presentations to subjects shaped for increases in the response were similar to the number of presentations to subjects shaped for decreases in response. However, it has been pointed out that the effects of the reinforcer on the autonomic response may itself cause differences between the increase and decrease groups in the number of reinforcements received over an operant training period.

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There is a real controversy when yoked controls are applied to studies of avoidance conditioning of autonomic responses. Crider, Schwartz and Shnidman (1969) have maintained that yoked controls are appropriate to such studies and that the criticisms of their use do not apply for avoidance conditioning. However, Katkin, Murray and Lachman (1969) maintain the position which regards yoked controls as unsuitable for all studies of operant autonomic conditioning. Despite this controversy, it is clear that since the effects of shock on the heart-rate response for animals in both the increase and decrease groups, in the present series of experiments, is always in one direction (i.e. a heart-rate increase) then a bias in favour of the yoked control for the appropriate heart-rate response exists for animals in both increase and decrease groups. The use of the yoked control group in the present series of experiments would, therefore, be supported. It could be argued by similar principles, that the use of the yoked control would only be justified if the classical conditioned component of the heart-rate response was unidirectional for all animals in both the increase and the decrease groups. Despite the absence of positive evidence for such homogeneity in the classical conditioned component, it was felt justified to compare experimental animals and their yoked controls, since it appeared that the unconditioned heart-rate response to shock was the primary variable which led to differences in the number of shocks received by animals in the increase and decrease groups.

EXPERIMENT

METHOD

Subjects

Ten hooded female rats weighing between 210-240 gm were taken from the colony of animals maintained in the Department of Psychology,

2. Footnote

The non-simultaneous yoked controls were employed partially because insufficient apparatus was available to run such controls at the same time as experimental animals. The presentations of stimulus (CS+) and shock to each experimental animals were determined by the heart-rate of that animal. However, for each yoked control, presentations of stimuli were given by manual operation of the programming apparatus by the experimenter. This was performed in such a way that the times of presentations of the shock and the stimulus (CS+) were almost exactly the same as had been the presentations of these stimuli to an experimental animal whose heart-rate had been shaped for either increases or decreases.

University of Durham, and used as the yoked controls. The experimental groups were comprised of a similar number of animals of comparable weight taken from the same colony. These experimental animals had undergone a sham operative procedure four days before they were used in the experiment, as the same animals were to serve as a control group in a later experiment. The operative procedure and appropriate experiment are described in the following chapter. These particular animals were chosen for this experiment because the effects of operant conditioning of heart-rate changes showed a difference in the response rates produced in the increase and decrease groups of rats and, simultaneously, a difference in the numbers of shock pulses received during the training schedule.

Apparatus

The apparatus for operant conditioning of heart-rate changes in the experimental animals has been described previously (Chapter Five). This same apparatus was also used for the yoked control animals, and the results from these animals were obtained after the results from the groups of experimental rats. This use of non-simultaneous yoked controls allowed the selection of the appropriate groups of experimental animals, which have been described and, consequently, a meaningful test of the hypothesis that the changes in heart-rate obtained by operant training procedures, within a bidirectional design, are not indirectly produced by differences in the numbers of shock pulses received by animals in the decrease and increase groups.[2]

Procedure for Operant Conditioning of Heart-Rate Changes

The experimental rats were curarised and artificially ventilated as described previously (see Chapter Five) Five of the animals were assigned to the increase group and were thus shaped to produce heart-

2. Footnote. Please turn over page.

rate increases. The remaining five animals were assigned to the decrease group in which it was attempted to shape a decrease in heart-rate. The procedures for these operant training schedules were also described in Chapter Five.

Each yoked control animal was 'paired' with an experimental rat which had previously been shaped for a specific heart-rate response. The procedure and the distribution of stimulus and shock presentations to the yoked animal was determined by the distribution of those variables to its paired experimental animal, and they were independent of its own heart-rate response.

RESULTS

The results of this experiment were again analysed in eight blocks of training trials for both the increase and the decrease groups of experimental animals and their yoked controls. There were no apparent differences between heart-rate responses during operant, CS+ and blank trials.

The change in the mean heart-rate response of each group of animals over successive blocks of trials is shown in Figure 6.6. This graph shows a divergence in the mean heart-rate response of the increase and decrease groups of experimental animals, whilst there appears little difference in the mean response rate of the comparative groups of yoked controls. The percentage change in heart-rate response over the training period for each experimental animal and its yoked control is presented in Table 6.B. A test for the difference in heart-rate response between the experimental increase and decrease groups gave a value $t = 2.8$, 8df; $p > .05 < .02$, and a similar test confirmed the absence of any difference between comparative groups of yoked controls ($t = 0.067$, 8df). The heart-rate responses which were obtained

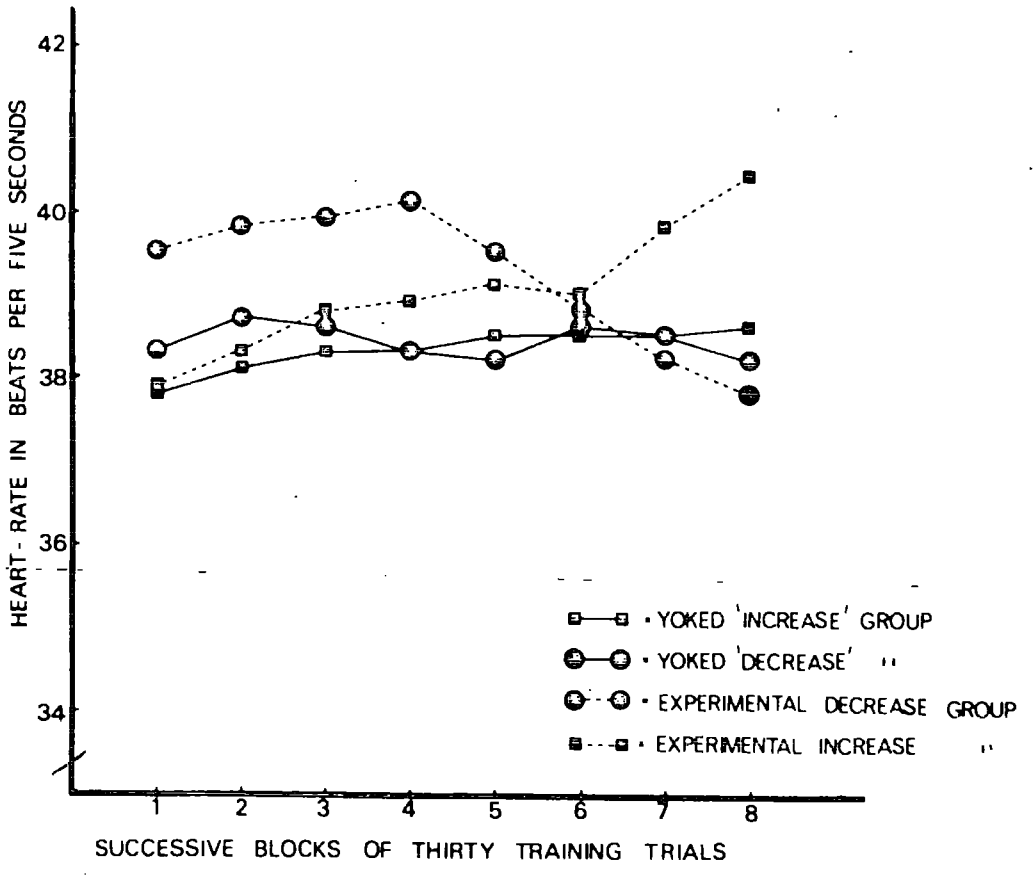

FIGURE 6.6 The mean changes in heart-rate over training of the experimental groups of curarised rats shaped for either increases or decreases in rate, together with the mean changes in rate for equivalent groups of yoked control animals. The graph shows a divergence of rates, in the appropriate directions, between the two groups of experimental animals, but an absence of a similar effect in the yoked controls.

TABLE 6.B The percentage change in heart-rate over training attained by each experimental animal and its yoked control, together with the frequency and probability of receiving shocks during training by that animal.

Column headings:

A = Percentage change in heart-rate over training trials attained by experimental animals. Negative numbers indicate a decrease in rate.

Z = Percentage change in heart-rate over training trials attained by yoked control animals. Negative numbers indicate a decrease in rate.

B = The mean number of shocks received per block of ten operant trials throughout training.

C = The mean number of trials per block of ten operant trials on which a shock was received during training.

	A	Z	B	C
INCREASE GROUP	10·3	2·2	11	4·4
	-1·5	-5·4	15·4	3·1
	17·7	3·6	17·6	3·3
	3·3	2·3	16·4	4
	17·8	4·9	12·8	2·3
DECREASE GROUP	5·3	-6·5	20·3	3·3
	-6·4	6·2	27·7	4·1
	-1·5	5·1	25·6	3·2
	-12·4	-5·6	22·8	2·2
	-5	-2·4	23·4	3·5

indicated that the changes in training procedure and the method of ventilation, suggested in the previous chapter, did lead to an increase in the success of showing decreases in the heart-rates of experimental animals shaped for that response.

The essential point of these results is that the absence of a difference between the heart-rates of the yoked control animals was found, despite a significant difference in the mean number of shocks received per block of operant trials by animals yoked to the increase and decrease groups ($t = 5.35$, 8df; $p > .001$). The contrast in the heart-rate responses obtained between the experimental animals and their yoked controls, even though they received the same frequency of shock pulses, can be seen in Figure 6.7, which presents a plot of the percentage change in heart-rate over the experimental period for each animal and, the mean number of shocks received per block of trials, by that animal. The mean number of shocks received per block of operant trials by each animal is presented in Table 6.B.

The increased effectiveness in producing appropriate decreases in heart-rate, by selecting lower levels for criterion shifts for animals in the decrease group, is supported by analysis of the data on the probability of receiving a shock on any operant trial. The mean number of trials per block of operant trials on which a shock was received is presented for each animal in Table 6.B. An analysis of the difference between the increase and the decrease groups for this parameter shows no significant effect ($t = 0.34$, 8df). This can be clearly seen from Figure 6.8 which presents a plot of this parameter of shock probability against the percentage change in heart-rate during the experiment for each animal.

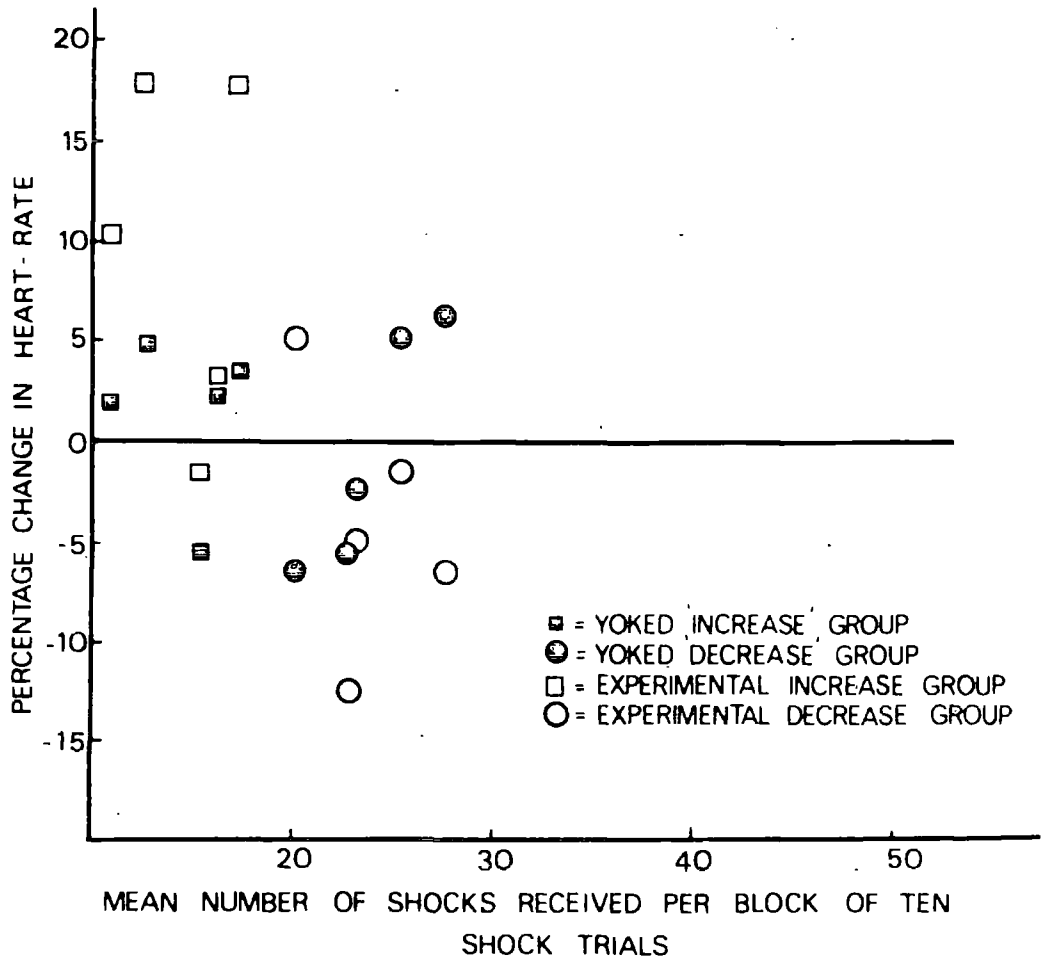

FIGURE 6.7 A plot of the mean number of shocks received per block of ten operant training trials, and the percentage change in heart-rate over the training period for each animal. The plot indicates that fewer shocks were received by rats shaped for heart-rate increases than by rats shaped for decreases.

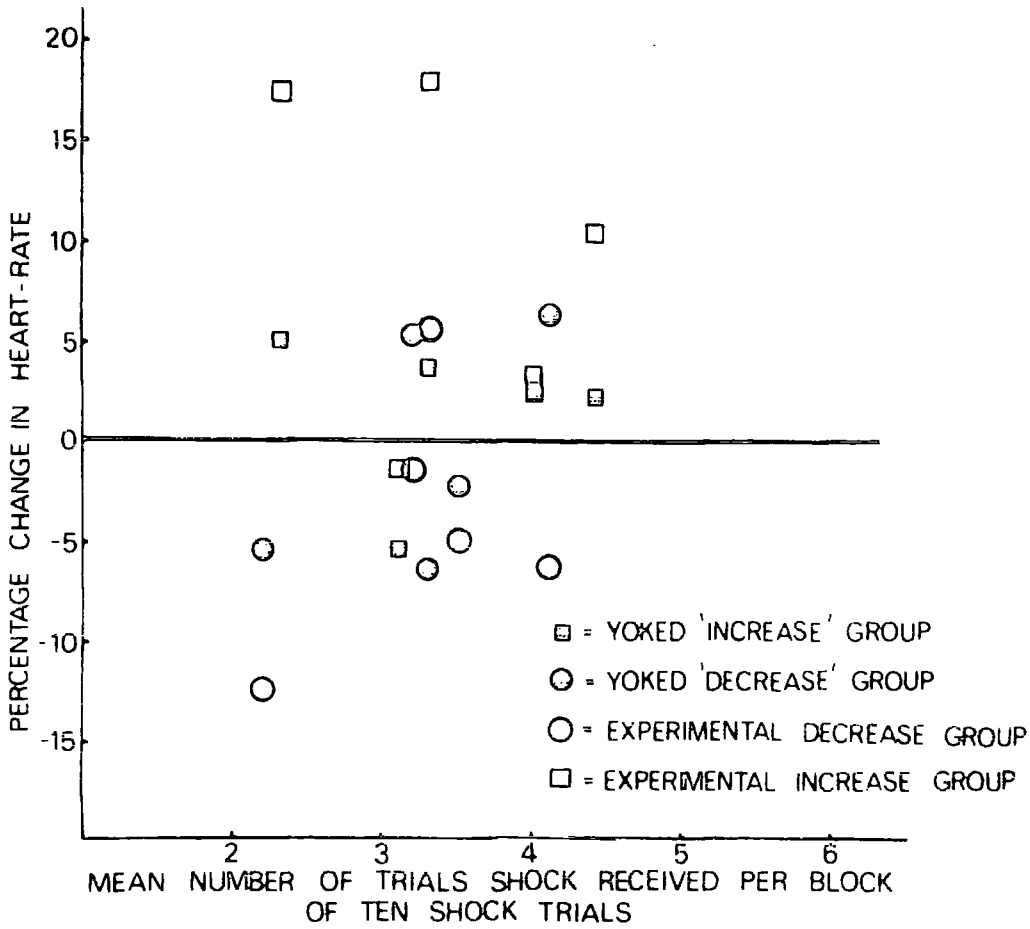

FIGURE 6.8 A plot of the mean number of trials per block of ten operant trials on which a shock was received by each animal, and the percentage change in heart-rate over the training period for that animal. There appears to be considerable overlap in the probability of receiving a shock on any one trial between animals shaped for increases or decreases in heart-rate.

DISCUSSION

These results confirm that the heart-rate changes produced by operant training schedule in the experimental animals were not an indirect consequence of the numbers of shock pulses received by each animal, but were produced by the operant contingencies.

The implied importance of operant contingencies in studies of cardiac conditioning has, itself, implications for the form of the heart-rate response of the yoked control, and the classical conditioned response, when an aversive unconditioned stimulus is used. If operant components are involved in such studies of classical heart-rate conditioning,then it would be predicted that a variable conditioned response should occur in the presence of the conditioned stimulus. When a short shock is used as the unconditioned stimulus, then heart-rate response during the conditioned stimulus, and immediately before the shock, would be punished and, consequently, since no response can consistently predict the presentation of the shock, a variable heart-rate would be produced. An alternative implication of operant components in studies of classical cardiac conditioning has been made by Zeaman and Wenger (1957) and Shearn (1961). They suggest that the form of the conditioned response is determined by the form of the heart-rate response at shock offset. However, their drive reduction interpretation encounters considerable difficulty in predicting the conditioned response when the unconditioned stimulus is a shock of brief duration. Limited data was obtained on the form of the classical conditioned cardiac response in the experimental situation used for the present studies on operant conditioning of heart-rate changes. Eight rats were curarised and artificially ventilated as in the experiments previously reported in this thesis. After a period of thirty minutes, during which the heart-rate was allowed to

stabilise, each animal was given fifty trials in which a five second conditioned stimulus (CS+) was followed by a shock pulse of 0.5mA for 0.1 seconds. The CS+ used was again the 60 watt lamp. Each trial was presented at variable intervals ranging from 20 to 50 seconds. Cardiotachometer recordings were obtained throughout the experiment for each animal, and these confirmed that the unconditioned response to shock on every trial, for each animal, was a heart-rate increase. However, the heart-rate of each animal during the CS+ was variable. On some trials this conditioned response was a heart-rate increase, on others it was a decrease, yet on other trials it was an increase followed by a decrease, and often there was no visible change in rate. Moreover, the conditioned response for each animal was not consistent on different trials. Although variability was expected on the hypothesis of the importance of operant components in studies of cardiac conditioning, a similar effect has been reported only twice in the literature. Black (1967) has shown that the classical conditioned cardiac response in the curarised dog is very variable when a brief shock is used as the unconditioned stimulus and Katcher, Solomon, Turner, Lolordo, Overmier and Rescorla (1969) have shown similar results in curarised dogs, when a variety of intensities and durations of shocks were used as the unconditioned stimuli.

It is uncertain whether the stable conditioned cardiac responses which are usually obtained in studies of classical conditioning in non-curarised organisms is indirectly produced by conditioning of skeletal responses. However, Yehle, Douth and Schneiderman (1967) using curarised rabbits, and Black and Lang (1965) using curarised dogs, have shown stable conditioned heart-rate responses during studies of classical conditioning, although it is unlikely that the level of curarisation was sufficient to suppress EMG activity of the animals in either experiment.

The importance of operant components in classical conditioning clearly requires further investigation, but the probable variability in the conditioned response would support those criticisms of the use of the yoked control in studies of operant conditioning of autonomic responses. However, in the present study, the primary use of the yoked animals was to control for the effects on heart-rate of shock frequency. Since it has been previously argued that it is the unconditioned cardiac component which is the major factor influencing this variable and, since this component is stable for all animals, then the conclusions from the use of the yoked controls are of value in supporting the hypothesis for operant manipulations of heart-rate changes.

CHAPTER SEVEN

OPERANT CONDITIONING OF HEART-RATE CHANGES IN BILATERAL ADRENAL DEMEDULLATED CURARISED RATS

In an earlier chapter it was pointed out that it is difficult to assess the physiological mechanisms responsible for the heart-rate changes obtained in studies of operant conditioning of specific rates of response. This is mainly due to the fact that such changes are assessed in terms of changes in baseline response rate and yet there is no knowledge about the baseline response levels of the autonomic nervous system under the conditions used in these studies. Earlier it was demonstrated that immunosympathectomised rats appeared to be unable to increase their baseline heart-rate response over a period of operant training for that response. It would be indefensible to conclude from that particular result that the increases in heart-rate obtained in normal rats, with a similar schedule, were mediated directly by sympathetic cardio-accelerator nerves. An alternative possible explanation was suggested for the result in terms of reduction in the range of the response with subsequent inability to maintain increase in heart-rate.

Adolph (1967) in a paper examining postnatal cardiac control in rats, has pointed out that small mammals have heart-rates that are tonically prodded. This concept suggests that rats are possibly over-invested with regard to their sympathetic nervous system and, consequently, it is unlikely that immunosympathectomised rats would show great deficits in normal levels of cardiac functioning. Van-Toller (1971) has suggested that deficits in behaviour of immunosympathectomised mice may be shown to occur if these animals are subjected to stress

(stress being defined as a departure from the normal physiological limits). Partially because of these studies, the question was pointedly asked as to whether the procedure for operant training in the curarised rat was highly stressful.

A diverse literature (Selye, 1950; Ramey and Goldstein, 1957; Von Euler, Genzell, levi and Strŏm, 1959) would support the work of Cannon (Cannon and de la Paz, 1911; Cannon, 1914, 1929; Cannon, Lewis and Britton, 1926, 1927; Cannon and Britton, 1927) which demonstrated that increased output from both the sympathetic nerves and the adrenal medulla produced increases in pulse rate and were important mediators of stress and emotion. The secretation from the medulla of the adrenal glands was called 'sympathin' by Cannon because of the similarity in its physiological effects to sympathetic activity. The presence of a catecholamine adrenaline, in adrenal medullae secretations partly accounted for the physiological effects produced.

In 1946, Von Euler showed that extracts from the adrenal glands also had properties resembling those of another catecholamine, noradrenaline. It is now known that both adrenaline and noradrenaline are stored in separate cells in the adrenal medulla (Hillarp and Hokfelt, 1953) and that their release is controlled by preganglionic sympathetic nerve fibres, running mainly in the splanchnic nerves. Douglas (1966) has pointed out that these medulla cells are the main stores of catecholamines in the body, although other extra-adrenal sources of similar chromaffin tissue have been identified (Vogt, 1960; Lempinen, 1964). Such studies would suggest that catecholamines from the adrenal medulla would play an important role for the maintenance of the range of response, in particular that of heart-rate, and a number of studies would support this hypothesis. Berti, Lentati

and Usardi (1965) demonstrated that if immunosympathectomised and adrenal demedullated rats were cold stressed, then their heart-rates were reduced to very low levels and eventually death occurred. Brody (1966), in an investigation of the vascular responses of immunosympathectomised rats, demonstrated that adrenal demedullation of these animals produced a considerable reduction in vascoconstrictor effects. A recent study by Carson (1970) is of interest, as it demonstrated that immunosympathectomised mice were unable to maintain high heart-rates even though they were able to show brief periods of tachycardia to shock stimulation. It was not determined if this brief tachycardia was reflecting differences in levels of circulatory catecholamines relative to normal controls, or whether it reflected more complex changes, both in vagal control of heart-rate and in the carotid sinus reflex as a possible result of chronic loss of cardiac sympathetic tone and chronic peripheral vascoconstriction. The additional effect of adrenal demedullation to these immunosympathectomised mice was minimal, lowering the baseline rates but not the brief response to shock stimulation. In the open field, however, where the stress was longer lasting, there was an increased effect on the heart-rate response, the adrenal demedullation producing even lower baseline rates. Although these studies suggest the importance of the adrenal medulla in the animal's adaptive range of response, they also point to the drastic procedures that are required to produce a reduction in the normal physiological response. They give no indication as to what degree of reduction in sympathico-medullary functioning is required to affect the sustained increases in heart-rate levels which are required in studies of operant conditioning presented in this thesis and which may, in themselves, be stressful.

More physiological studies provide less convincing evidence for the suggested effects of demedullation on the heart-rate response. Von Euler (1956) has shown that noradrenaline is a normal constituent of the mammalian heart, where it is stored in adrenergic nerve endings in dense core vesicles. It has been assumed that, since the heart can take up large amounts of noradrenaline, the catecholamines discharged into the blood stream from the adrenal medulla and sympathetic nerves (as a result of normal flow of tonic impulses from the central nervous system) could contribute to the maintenance of transmitter stores in the adrenergic nerve vesicles. However, more recently it has been shown that the myocardium contains all the necessary enzymes for the synthesis of noradrenaline from its dietary precursors (Levitt, Spector, Sjoerdsma and Udenfriend, 1965) and, consequently, the maintenance of myocardial catecholamines may not be totally dependent on the uptake of catecholamines of adrenal origin (Bhagat, 1963; Bhagat and Shideman, 1964; Avakian and Vogt, 1966; Borchard and Vogt, 1970). Kopin and Gordon (1963) have calculated that, in the intact animal, 80 percent of the noradrenaline in the heart is synthesised in cardiac tissue, whilst only 20 percent is taken up from circulating amine, and it is probable that not all of this circulating amine is of adrenal origin. Moreover, since increased sympathetic activity results in increased synthesis of noradrenaline (Bhagat, 1967; Bhagat and Friedman, 1969) then it seems logical that cardiac catecholamine levels could be maintained after the removal of the adrenal medulla by increased catecholamine synthesis in peripheral sympathetic neurones. Westfall and Osada (1968) have confirmed that increased sympathetic nerve activity to the heart could compensate for the effects of adrenalectomy and so maintain tissue levels of noradrenaline. Bhagat (1969) has

also shown no reduction in the cardiac levels of noradrenaline after adrenal demedullation.

In contrast to the more behavioural studies of Berti et al (1965), Brody (1966) and Carson (1970), the physiological evidence, primarily amassed by Bhagat and his associates, would indicate that adrenal demedullation would have a minimal effect on the adaptive range of cardiac response, though concurring with the importance of sympathetic cardiac nerves. Celander (1955), in a monograph analysing the range of control exercised by the sympathico-adrenal system, had indicated that, in his experiments, the adrenal medulla played a limited role in facilitating evoked responses in the sympathetic nervous system.

In an attempt to assess the importance of adrenal medullae secretions to baseline heart-rate levels under the experimental conditions of studies presented for this thesis, an attempt was made to shape heart-rate changes in the bilateral adrenal demedullated, curarised rat.

EXPERIMENT

METHOD

Subjects

Two litters of neonatal rats were obtained from the Department of Psychology, University of Durham. The first litter of sixteen hooded rats were used as the experimental groups and they were adrenal demedullated when weighing between 190-200 gm. The ten hooded rats from the second litter were used as sham-operated controls when they weighed between 210-250 gm.

Operative Procedures.

Bilateral adrenal demedullations and the sham-operations were performed under Nembutal anaesthesia (40 mg/Kgm body weight, injected intraperitoneally). The bilateral demedullation was performed in a single-stage operation. The adrenal glands were exposed through incisions on either side of the abdomen as described by Ingle and Griffith (1963). Each adrenal cortex was then slit and the medulla extruded by gentle squeezing of the gland with forceps. The incisions were then closed with surgical stitches and the wounds treated with sulphonamide powder to protect against infection.

Non-demedullated sham-operations involved a similar procedure but the adrenal glands were merely exposed and disturbed with forceps before the incisions were closed. In addition a 2 cm sagittal incision was made in the scalp and, after retracting the skin, the peritoneum scraped from the skull. The wound was then closed and treated with sulphonamide powder. This joint procedure was an attempt to control for the operative procedure of adrenal demedullation and also the general surgery involved in the procedure for producing cortical spreading depression (see Chapter Six).

Animals in both experimental and sham control groups were given a subcutaneous injection of penicillin (6,000 units) following the operative procedure. Operant heart-rate training procedures were initiated five days after the operative procedures.

Procedure for Operant Conditioning of Heart-Rate Changes.

The procedure for curarisation, artificial ventilation and operant conditioning of heart-rate changes has been described previously (see Chapter Five). Half of the animals (8) in the experimental group and half of the animals (5) in the sham-operated control group were

shaped for increases in their heart-rates, whilst the remaining animals were shaped for decreases in their heart-rates. Unfortunately, data from one experimental animal which was being shaped for heart-rate increases was lost due to a failure in the programming apparatus.

RESULTS

The results were again analysed in eight blocks of training trials. As in previous experiments there were no differences in the levels of heart-rate responding between operant, CS+ and blank trials.

The group mean changes in heart-rate response over the successive blocks of operant training trials for the demedullated and control animals are, respectively, shown in Figures 7.1 and 7.2. It would appear that there is little difference between the response of demedullated animals shaped for heart-rate increases and the heart-rate response of the similar experimental group shaped for heart-rate decreases. This effect is a contrast to the changes in response rates observed in the two sham-operated control groups, in which a clear discrepancy is seen between the increase and the decrease groups. The percentage change in heart-rate during operant trials over training, for each adrenal demedullated rat is presented in Table 7.A, and the changes of rate for the sham-operated controls over a similar period, is presented in Table 7.B. A t-test confirms the absence of a difference in heart-rate responses between the increase and decrease groups of experimental demedullated animals ($t = 0.23$, 13 df). A similar test for the difference between control animals gives a value of $t = 2.8$, 8df; $p > .05 < .02$).

The mean number of shocks received per block of operant trials by each experimental, demedullated rat is given in Table 7.A. There

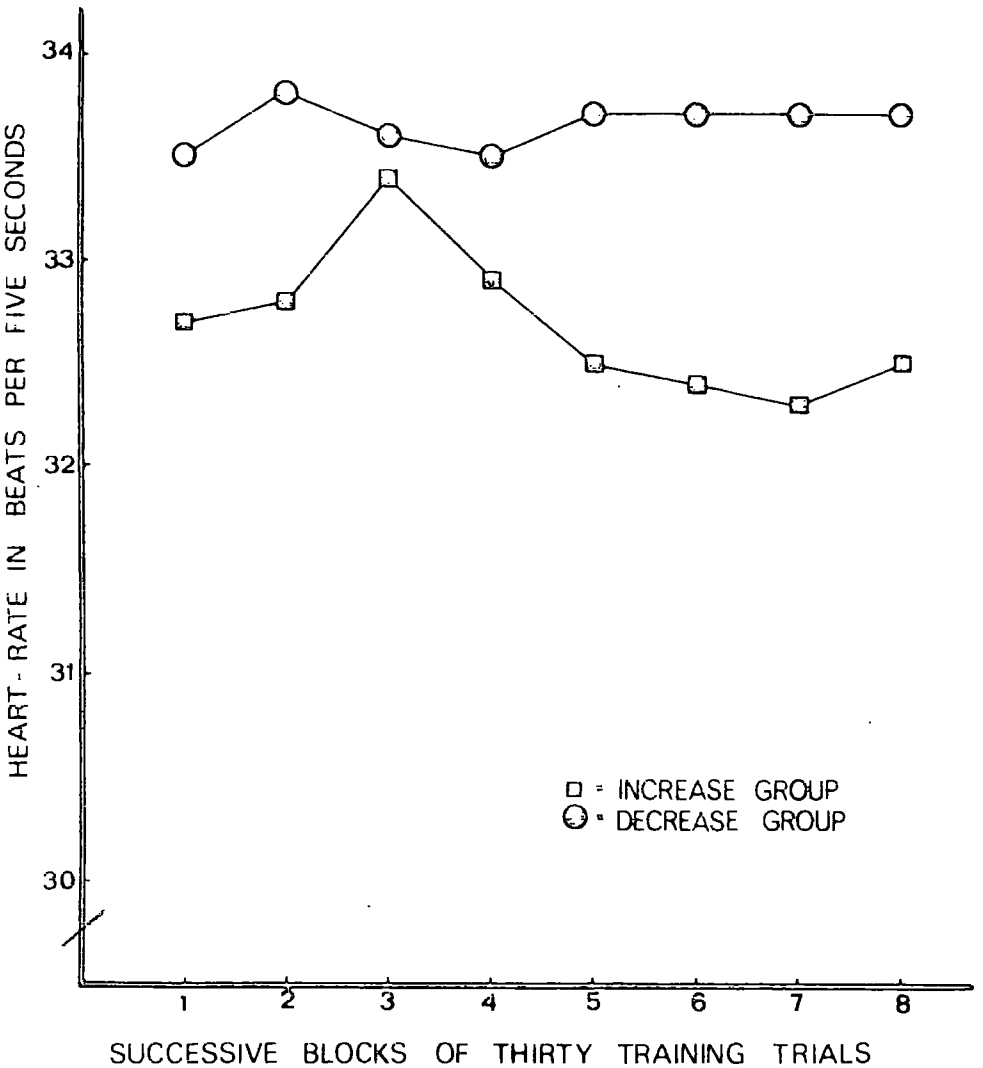

FIGURE 7.1 The mean changes in heart-rate, over successive blocks of trials, of groups of bilateral adrenal demedullated curarised rats shaped for either increases or decreases in rate. Both groups appear to show little change in the mean heart-rate response over the training period.

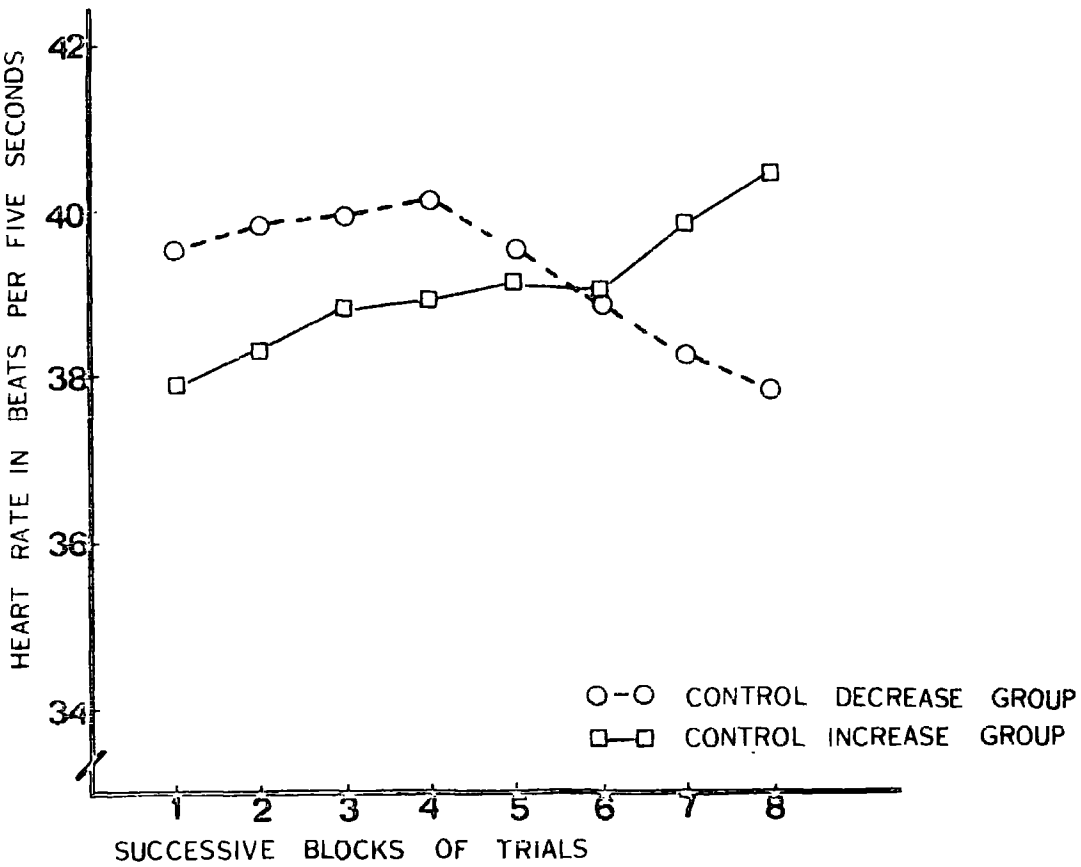

FIGURE 7.2 The mean changes in heart-rate over successive blocks of trials of control rats shaped for either increases or decreases in rate. The mean heart-rate response of the two groups appears to diverge over the training period, each group showing the appropriate change in direction of response suggested by the operant contingencies.

TABLE 7.A The percentage change in heart-rate over training attained by each bilateral adrenal demedullated curarised rat together with the frequency with which shocks were received during training by that animal.

Column headings:

A = Percentage change in heart-rate over training trials. Negative numbers indicate a decrease in rate.

B = The mean number of shocks received per block of ten operant trials throughout training.

INCREASE GROUP			DECREASE GROUP		
SUBJECT	A	B	SUBJECT	A	B
1	1.1	23.5	1	7.3	28.1
2	-13.1	19.6	2	-6.0	18.4
3	7.1	12.4	3	19.0	29.5
4	-6.9	29.8	4	-0.5	23.3
5	-4.6	22.1	5	-4.7	17.9
6	6.3	16.0	6	0	10.8
7	8.4	18.9	7	-9.3	22.6
8	N O D A T A		8	0.5	24.8

TABLE 7.B The percentage change in heart-rate over training attained by each control animal, together with the frequency with which shocks were received during training by that animal.

Column headings:

A = Percentage change in heart-rate over training trials. Negative numbers indicate a decrease in rate.

B = The mean number of shocks received per block of ten operant trials throughout training.

INCREASE GROUP			DECREASE GROUP		
SUBJECT	A	B	SUBJECT	A	B
1	10.3	11	1	5.3	20.3
2	-1.5	15.4	2	-6.4	27.7
3	17.7	17.6	3	-1.5	25.6
4	3.3	16.4	4	-12.4	22.8
5	17.8	12.8	5	-5	23.4

is no difference between the increase and decrease groups of these animals in this parameter of shock frequency ($t = 0.62$, 13df) and this can be clearly seen in Figure 7.3, which presents a plot of the percentage change in heart-rate over training attained by each animal and the mean number of shocks which were received per block of operant trials by that animal. The values of the same parameter of shock frequency for sham-operated control rats are presented in Table 7.B. A test for the difference between the increase and decrease groups of these animals gave a value of $t = 5.35$, 8df; $p > .001$. This difference is clearly apparent in Figure 7.4 which presents a plot of the mean number of shocks received per block of operant trials by each control animal, and the percentage change in the heart-rate over training for that animal.

DISCUSSION

The results indicate that it was difficult to demonstrate operant conditioning of increases or decreases of heart-rate in bilateral adrenal demedullated curarised rats. The absence of a differential effect between the increase and decrease group of animals is a contrast to the results obtained from the sham-operated animals. Divergence between the two groups of control animals in their heart-rate was produced by increases in rate for animals shaped for that response, and by decreases in heart-rate for animals in the decrease group. The heart-rate changes obtained could be considered as support for the hypothesis that adrenal demedullation reduced the 'adaptive range of response', resulting in an inability to sustain the increases in baseline heart-rate response which are required by the operant training schedule. The

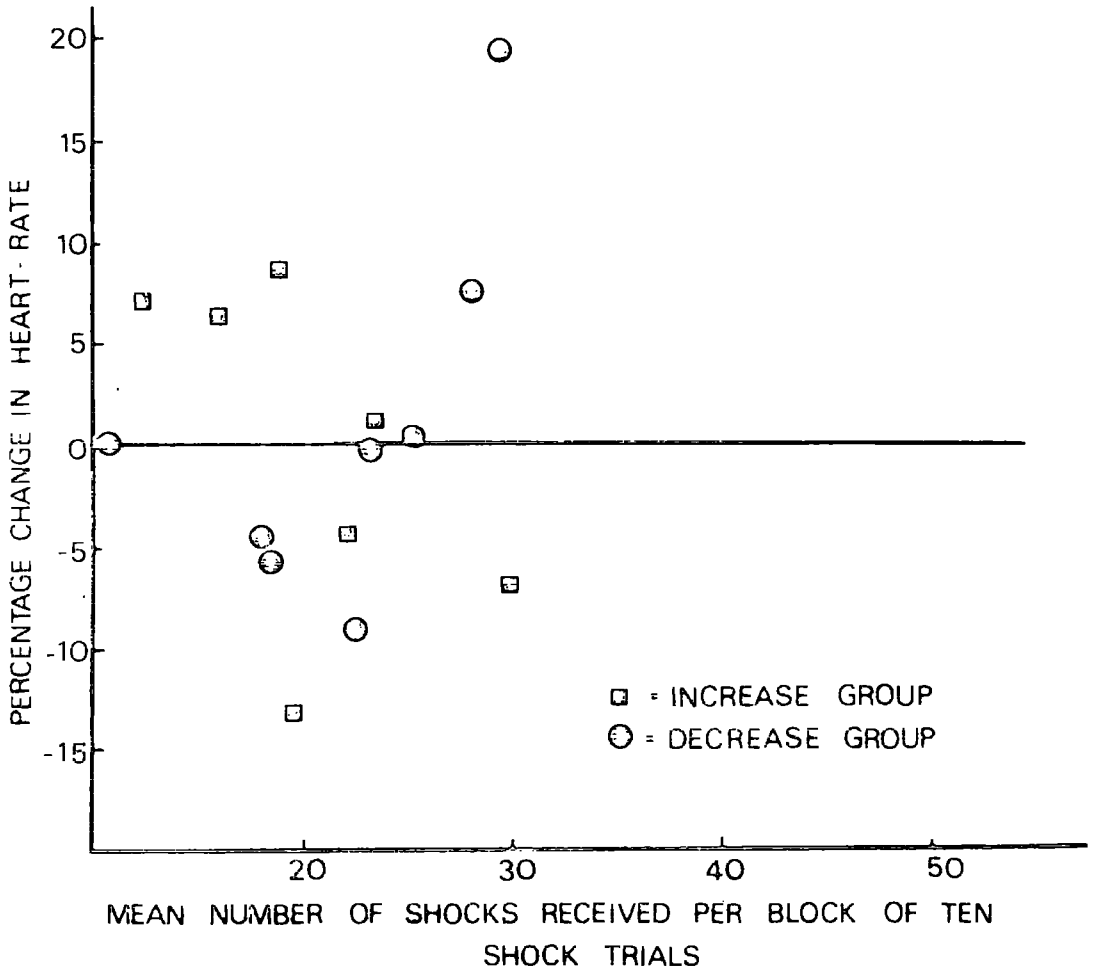

FIGURE 7.3 A plot of the mean number of shocks received per block of ten operant trials, and the percentage change in heart-rate over the training period, for each adrenal demedullated animal. There is considerable overlap in the numbers of shocks received during training by animals in the increase and decrease groups.

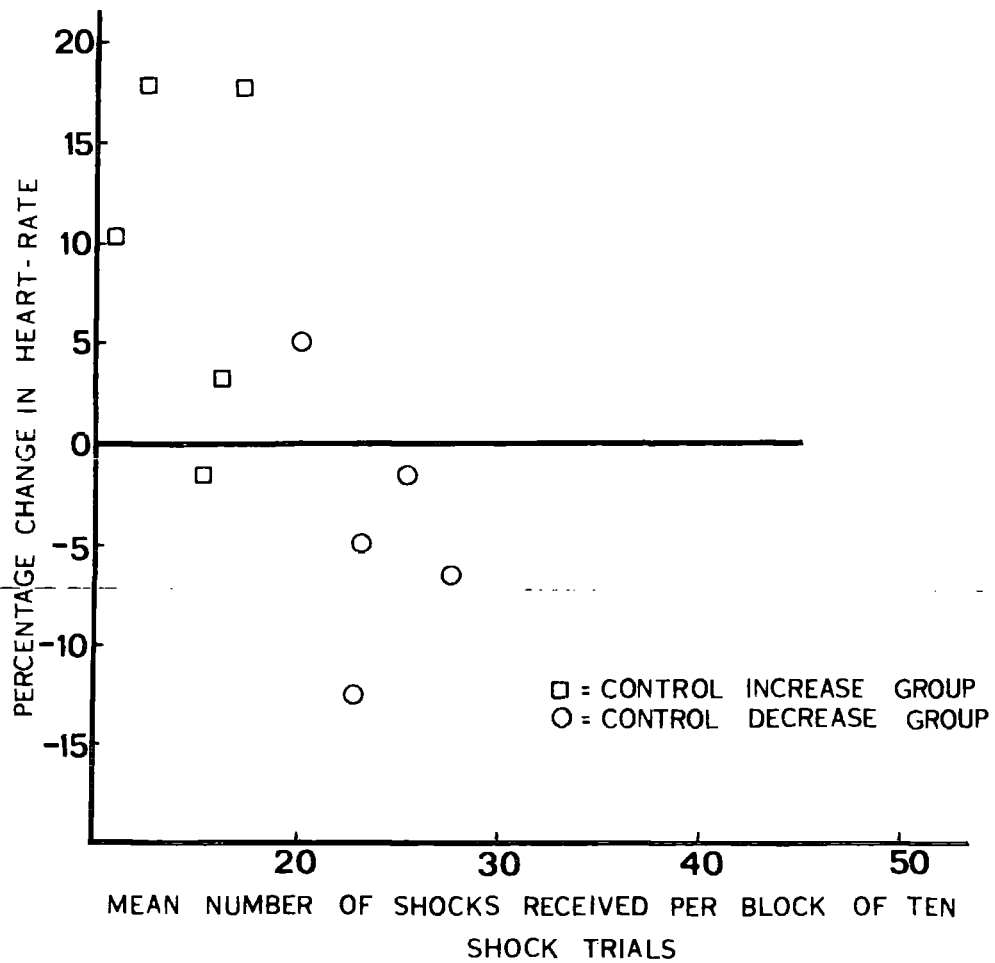

FIGURE 7.4 A plot of the mean number of shocks received per block of ten operant trials, and the percentage change in heart-rate over the training period for each control animal. The plot indicates that control animals shaped for increases in heart-rate received considerably fewer shocks than animals shaped for decreases in rate.

absence of any success in shaping decreases in heart-rates of experimental adrenal demedullated rats was unexpected. The body weights of the experimental rats were deliberately chosen to be lower than those of rats in the other studies, since young rats have only small deposits of fatty tissue and, consequently, extra-adrenal chromaffin tissue. It may have been that the conditions of ventilation for the experimental animals were, subsequently, not conducive to decreases in baseline heart-rate levels. The result emphasizes the danger in interpretation of specific changes in heart-rate in the curarised animal and the need to compare animals, which presumably require similar conditions for adequate ventilation, within the bidirectional design.

It could be argued that the absence of differential heart-rate changes between the increase and decrease groups of adrenal demedullated rats was a consequence of the absence of a difference between these groups in the numbers of shocks received by them. This argument is supported by the fact that a significant difference was found between the increase and decrease groups of sham-operated control animals in the number of shocks received as well as a difference in their heart-rate responses. However, the experimental evidence presented in the previous chapter would not support this suggestion of a close relationship between heart-rate changes and the parameter of shock frequency. The reduced adaptive range of response produced by adrenal demedullation could also explain this result of an absence in the difference in the numbers of shocks received by the increase and decrease groups of experimental animals. It has been suggested that the mild tachycardia which occurred as the unconditioned response to shock would favour animals in the increase group in the probability of escape on any

one operant trial and, consequently, it would be expected that increase group animals would receive less shocks during training. Since such unconditioned increases in heart-rate were observed from the cardiotachometer records of adrenal demedullated rats, the overlap in numbers of shocks received by the experimental animals in both groups was unexpected. When an account is taken of the possibility that adrenal demedullated animals may not be able to maintain increases in heart-rate, and that criterion level shifts for animals in the increase group are greater than for the decrease group, then an explanation is readily seen. A demedullated rat in the increase group, whose heart-rate had successfully attained a low criterion level, would, on a shift to higher criterion level, be unable to maintain the previous heart-rate level and, consequently, would receive many shock impulses on that trial.

The results of this experiment are somewhat surprising, since the introductory comments of this chapter leads one to expect that a reduced ability to maintain increases of heart-rate in adrenal demedullated rats would be found only under extreme conditions of sympathetic functioning or stress. The possibility that the experimental procedure used in these experiments on operant conditioning of heart-rate changes is highly stressful is considered in some detail in the following chapter. It would seem that both the sympathetic cardiac nerves and adrenal medullae contribute to the increases in heart-rate which have been obtained in these studies. It is likely that such a conclusion may hold only for operant conditioning studies in which curarised animals are used, and generalisation of those results to possible studies on operant conditioning of heart-rate changes in the non-curarised state is not justified.

CHAPTER EIGHT

CATECHOLAMINE METABOLISM IN THE CURARISED RAT

The results of experiments presented in previous chapters would indicate that the curarised rat may be in a stressful situation, involving high sympathetic activity, and this is probably augmented by operant training procedures in which a negative reinforcer is involved. Support for this hypothesis can be indirectly claimed from the study by Koelle (1965) which demonstrated that the output of the sympathico-medulla system was increased in the curarised animal to compensate for changes in circulatory dynamics that the drug produced.

If the curarised animal is in a stressful situation then, not only would manipulations of the sympathico-medullar system affect the ability to increase heart-rate, but also there would be an expected change in peripheral catecholamine levels as a result of increased baseline sympathetic activity. This conclusion is supported by the studies of Sigg, Day and Columbo (1966) who have shown increases in noradrenaline levels of the spleen and adrenals when mice were subjected to isolation stress. Moore (1968) similarly reported increases of catecholamine levels in the urine of chronically isolated rats. An experiment by Mason, Tolsen, Brady, Tolliver and Gilmore (1968) is of special interest, since they demonstrated marked catecholamine output from monkeys under a stressful situation in which there was an element of unpredictability or uncertainty, in combination with the threat of a noxious stimulus, and with the involvement or 'trying' in the performance of a self protective task. These elements are very similar to those encountered by the rat in

studies of operant conditioning of heart-rate changes which have been presented in this thesis.

Other studies have indicated that there may be depletion of catecholamines, or a reduction in the adaptive range of response, if the stress is prolonged or made severe. Johnson, Schönbaum and Sellers (1966) have shown that immunosympathectomised rats had considerable reserves of catecholamines when subjected to cold stress, but these proved inadequate when the stress was made more severe by clipping the fur. Gordon, Spector, Sjöerdsma and Udenfriend (1966) have reported reductions in the levels of adrenaline in the adrenal glands of rats after sustained periods of exercise and similar, but less acute, reductions after cold stress. Interesting studies have shown that brief periods of fighting in mice produce large increases in adrenal catecholamines but, if fighting is prolonged, these catecholamine levels, especially the adrenaline levels, are markedly reduced (Welch and Welch, 1969a, 1969b).

Despite the focus on peripheral catecholamine metabolism, there have been a number of studies which indicate changes in brain levels of catecholamines during affective or stressful conditions. Barchas and Freedman (1963) have demonstrated that there was considerable depletion of total brain levels of noradrenaline when rats were swum to exhaustion in cold water, although cold stress alone produced considerably less effect. Electric shocks to the feet of rats and guinea pigs have been shown to produce considerable and rapid depletion of endogenous brain levels of noradrenaline (Maynert and Levi, 1964). Bliss and Zwanziger (1966) considered the effects of immobilisation stress and electric shocks on brain levels of noradrenaline in rats, mice and guinea pigs, and found considerable decrements in these levels in various regions of the brain which did not appear to

be produced by changes in muscular activity. Thierry, Javoy, Glowinski and Kety (1968) found that the effects of electric shock on endogenous brain levels of noradrenaline produced depletion only if stimulations of high intensity were used. Mild shocks produced no depletion, although a marked increase in the turnover of noradrenaline did occur. These effects of electric shock stress appeared mainly in the brainstem-mesencephalon areas of the brain. Mice exposed to the stress of aggression and defeat for short periods would appear to have increased levels of noradrenaline in the hypothalamus and amygdala, but if the period was extended these levels were considerably depleted (Eleftheriou and Church, 1968). This latter result has been supported by the studies of Welch and Welch (1969a, 1969b), which indicate that, although brief periods of fighting in mice produced increases in levels of noradrenaline in the brainstem, more prolonged fighting produced considerable depletion of these levels.

These studies could be summarised as emphasizing that a variety of acute emotional stresses, including electric shocks, produced depletion in brain levels of noradrenaline which appear most markedly in the brainstem regions. Two other studies have particular interest in relation to this thesis. The first was performed by Sanan and Vogt (1962) and showed that there was a high correlation between reduction in brain levels of noradrenaline, reduction in adrenal catecholamines, and also high sympathetic nervous activity. The importance of their study is evident since it has been suggested that the conditions of the experiments in this thesis produce high sympathetic activity, and hence changes in catecholamine levels of the adrenals and brain would be expected. The second study, by Jacobsen and Redfern (1970), has indicated increases in the levels of noradrenaline in the lower brainstem of spontaneously hypertensive rats and, consequently, suggests an intimate relationship between activity of the peripheral circulation and known central cardiac control centres.

3. Footnote

The study which was undertaken was intended only as a preliminary investigation of the effects of curarisation procedures on the levels of catecholamines in the adrenals, heart and lower brainstem of the rat. The results of the study were disappointing because of the heterogeneity in the tissue levels of the catecholamines as indicated by the technique of analysis used for their assay. This heterogeneity in the data, together with a low percentage recovery of noradrenaline from the tissues, would indicate possible inadequacies in the fluorometric assay method which was used. However, during storage of the tissues a number of cuts in electricity supply were reported to have occurred, and it may be that degeneration, or more importantly, differential degeneration, of tissue catecholamines occurred during cold storage. Consequently, the quantitative nature of the results being reported in this study should be regarded as being of tentative value.

EXPERIMENT

INTRODUCTION

As a consequence of the considerable number of studies which have shown an intimate relationship between central and peripheral levels of catecholamines and stressful situations involving high sympathetic output, an attempt was made to ascertain the peripheral catecholamine output and the endogenous levels of noradrenaline in the lower brainstem produced by the baseline experimental conditions involved in the present series of experiments on operant conditioning of heart-rate changes.

Increased sophistication has allowed for measurement of peripheral levels of catecholamines in a variety of ways. Analysis of urinary levels of catecholamines reveals only a small percentage of the total output of the adrenals because amines released into the blood are rapidly broken down. Assessment from blood samples was impossible, since the animal was not to be disturbed. The alternative was to measure levels directly in the tissue after the experiment, and this was undertaken for both the adrenals and the heart, as these were the main organs on which interest was focused. A similar procedure was used for an assay of levels of noradrenaline in the brainstem.[3]

METHOD

Subjects

Thirty male hooded rats weighing between 190-300 gm were taken from the colony of animals maintained in the Department of Psychology, University of Durham, and each animal was randomly assigned to one of three groups.

3. Footnote. Please turn over page

Group 1. Animals were curarised and artificially ventilated in the same apparatus and in the same manner as described in previous experiments. The animals remained in the time-out conditions of total darkness for the whole experimental period of $2\frac{1}{2}$ hours.

Group 2. Animals in this group were curarised and artificially ventilated in a similar manner to rats in Group 1. However, after a period of thirty minutes in time-out conditions, they were presented with a schedule of light and light-shock stimulations. The schedule of this series of stimulations was determined from the schedule of presentation to other rats which had undergone operant training of heart-rate changes in previous experiments. The schedule was, therefore, independent of the rat's own heart-rate.

After a period of $2\frac{1}{2}$ hours under experimental conditions, animals from both Groups 1 and 2 were killed.

Group 3. Group 3 was a control group in which animals received no experimental treatment, but merely individually taken from the home cage and immediately killed.

Assay Procedure.

Immediately after killing each animal, the adrenals and the heart were successively dissected out as quickly as possible, cleaned of fat and connective tissue, rinsed in ice-cold isotonic saline, and then frozen on the side of a beaker immersed in a mixture of alcohol and cardice. After these procedures, the brain was dissected out, the cerebellum removed and the medulla-hindbrain (lower brainstem) divided off with a cold scalpel and frozen on the side of the beaker with the heart and adrenals. After freezing, the

separate weights of the heart, the lower brainstem and the combined adrenal glands were noted. The adrenals were stored at -15°C overnight and assayed on the following day. The heart and lower brainstem were placed in a labelled tube and stored at -15°C until they were assayed five months later.

The catecholamine assay technique used for the adrenals and noradrenaline estimation of the adrenals was a differential pH trihyroxyindole fluorometric method, using potassium ferricyanide as the oxidant. Noradrenaline content estimations were made on the other tissues by precipitating the protein material and making a chromatographic separation of the noradrenaline using small resin columns. Following the resin separation, a trihydroxyindole fluorometric estimation of the noradrenaline was made. Full details of the assay method used are presented in the Appendix.

Experimental Procedure and Design.

The length of time required for the experimental procedures of Groups 1 and 2 limited the number of animals which could be used on each day. Since analysis of adrenal catecholamine levels must proceed on the day following the death of an animal, the procedures for the three groups of rats were carried out over four separate occasions. The number of animals used from each group was the same on each day. On the first two days, tissues were obtained from two animals in each group, and on the final two days, the number was increased to three animals in each group.

The noradrenaline levels in the heart and lower brainstem of all the rats were ascertained from only two separate analyses, one for each tissue. To do this, it was necessary to bulk similar

tissues into units of two. The tissues for each unit were chosen at random from within the same treatment group of animals.

RESULTS

The total body weights and the weights of the various organs of animals in each experimental group are presented in Table 8.A. Critical ratios computed for the means of body and tissue weights failed to show any significant differences between any of the experimental groups.

Adrenal Catecholamine Assay.

The results of the catecholamine assays on the adrenal tissue are presented in Table 8.B and 8.C. The tabulated values of both noradrenaline (Table 8.B) and adrenaline (Table 8.C) are expressed as ugm/gm of adrenal tissue. These values of noradrenaline are very similar to the levels of noradrenaline in the adrenals of the rat which have been reported in other studies (Gordon, Spector, Sjoerdsma and Udenfriend, 1966; Moore, 1968). The values for adrenaline, however, appear to be only 25.33 percent of the levels reported in the literature (Eränkö and Räisänen, 1957; Moore, 1968). The difference in techniques in these various studies for assaying catecholamine levels is considerable, and it may be that the differential pH trihydroxyindole fluorometric analysis suggests lower levels of adrenaline. This factor was unimportant in the present study, since it was the relative level of catecholamines in different experimental groups which were of interest.

Because of the difference in the numbers of tissues assayed on each occasion, and also the heterogeneity in the levels of catecholamines, in particular the levels of noradrenaline, the results

TABLE 8.A The body and organ weights of the three experimental groups of rats.

		Group 1	Group 2	Group 3
Body weight (gm)	Mean SE	247.2 ± 11.5	251.2 ± 11.09	241.5 ± 11.43
Weight of pair of adrenals (gm)	Mean SE	0.0522 ± .0078	0.0491 ± .0043	0.0539 ± .0036
Weight of heart (gm)	Mean SE	1.750 ± .047	1.802 ± .072	1.675 ± .048
Weight of lower brainstem (gm)	Mean SE	0.5091 ± .024	0.5337 ± .034	0.5148 ± .0071

TABLE 8.B The noradrenaline content of the adrenals from individual rats in the three experimental treatment groups. The values are expressed as ugm/gm of adrenal tissue, and were obtained from different animals on four separate days. Note the heterogeneity in values obtained during the separate assays.

	Group 1	Group 2	Group 3
Assay A	20.61 16.01	116.30 99.50	74.24 50.53
Assay B	19.7 26.9	34.02 8.58	0 0
Assay C	22.99 24.28 16.64	22.19 16.49 11.71	20.76 18.02 42.64
Assay D	25.58 11.69 14.98	14.53 49.34 20.96	24.98 10.53 18.95
Mean SE Median	18.94 $\pm$ 2.20 20.61	39.34 $\pm$ 11.46 21.59	26.01 $\pm$ 7.04 19.86

TABLE 8.C The adrenaline content of the adrenals from individual rats in the three experimental treatment groups. The values are expressed as ugm/gm of adrenal tissue, and were obtained from different animals on four separate days.

	Group 1	Group 2	Group 3
Assay A	35.10 58.69	66.14 35.28	79.52 79.07
Assay B	55.88 54.95	53.76 54.88	91.10 77.60
Assay C	75.81 53.93 69.72	72.74 58.66 67.73	78.31 75.28 79.65
Assay D	62.64 44.22 49.11	74.08 41.92 63.26	58.92 66.78 77.26
Mean SE Median	56.00 ± 3.55 54.55	58.85 ± 3.82 60.96	76.35 ± 2.55 78.92

were analysed by non-parametric statistics. The results obtained from the assays were subjected to a Kruskal-Wallis one-way analysis of variance by ranks, to decide whether there was any difference in levels of each catecholamine between the three experimental groups. A further analysis was used in order to test not only this main effect of difference between the experimental groups, but also to confirm the absence of any possible, though unlikely, interaction between the experimental groups and the four separate assays. The appropriate test was suggested by Bradley (1968) and involves a simple modification of the Kruskal-Wallis analysis. Where their analysis indicated a significant level, a Mann-Whitney analysis was carried out to test the possible difference between each group.

Adrenaline Content of the Adrenals.

The initial Kruskal-Wallis one-way analysis of variance revealed a value of $H = 16.48$, $df = 2$; $p > .001$, which indicated that the adrenaline content of the adrenals differed significantly in rats from the three experimental groups. The analysis, suggested by Bradley (1968), confirmed this result as the test for the main effect of the experimental group gave a value $H = 7.53$, $df = 2$; $p > .02$. The second main effect of performing assays on four separate occasions had no significant effect ($H = 3.94$, $df = 2$). Moreover, the analysis confirmed that there was no significant interaction between the levels of adrenaline in the different experimental groups, and the effect of the four assays ($H = 9.42$, $df = 6$). Mann-Whitney tests for the differences between the experimental groups revealed two significant results. The levels of adrenaline in the adrenals from rats in the control group, Group 3, appeared to be significantly higher than comparative levels of

rats from Group 2 (U = 8, one-tailed test significant at .001 level) and the levels in the control groups were also higher than levels from rats in Group 1 (U = 6, one-tailed test significant at .001 level).

Noradrenaline Content of the Adrenals.

The initial Kruskal-Wallis one-way analysis of variance indicated no significant difference between the three experimental groups in noradrenaline levels of the adrenals (H = 2.5, df = 2). Moreover, this was confirmed by the more stringent analysis for the main effect of the experimental groups (H = 1.42, df = 2). The second main effect, of the separate assays, was significant (H = 6.85, df = 2; $p > .02$). This can be clearly seen in Table 8.B which presents the levels of noradrenaline in ugm/gm of adrenal tissue for each of the three experimental groups on each separate assay. The cause of this heterogeneity between separate assays was unclear and could have been produced by several unidentified variables in the assay procedure. However, this result does not affect the conclusion that there were no apparent differences between the three experimental groups in levels of adrenal noradrenaline, since the interaction between this main effect and the main effect of separate assays was not significant (H = 11.4, df = 6).

Noradrenaline Content of the Lower Brainstem.

The noradrenaline levels in the pairs of bulked lower brainstems of rats in the three experimental groups is presented in Table 8.D. These levels of noradrenaline were within the ranges which have been

TABLE 8.D The noradrenaline content of lower brainstems from pairs of rats in the three experimental treatment groups. Values are expressed as ugm/gm of tissue.

	Group 1	Group 2	Group 3
	0.642	0.497	0.838
	0.456	0.431	1.088
	0.107	0.775	0.722
	0.633	0.417	0.603
	0.299	0.467	0.601
Mean	0.427	0.517	0.770
SE	± .102	± .064	± .0900
Median	0.456	0.467	0.722

obtained in other studies (Maynert and Levi, 1964; Bliss and Zwanziger, 1966; Gordon, Spector, Sjoerdsma and Udenfriend , 1966; Thierry, Javoy, Glowinski and Kety, 1968; Jacobsen and Redfern, 1970).

Iversen (1967) has indicated that tissues may be stored for considerable periods of several weeks at low temperature with little degeneration of catecholamines. It would appear that a similar absence of any depletion of noradrenaline in the lower brainstem occurred in the present study in which tissues were stored at $-15^{\circ}C$ for a longer period of five months. However, in anticipation of the data (given below) for levels of noradrenaline in the heart, there was likely to be some depletion in noradrenaline level, the extent of which was not ascertained in the present study. Moreover, in the assay technique used in this laboratory, the recovery rate from the internal standards of noradrenaline run through the resin columns was not high. In view of these considerations, the results presented in Table 8.D should be regarded as a qualitative, rather than a quantitative, record.

The introductory comments in this Chapter suggested that the levels of noradrenaline in the brainstem of rats which were stressed, would be lower than control animals. Specifically, the hypothesis was offered that these levels would, therefore, be reduced in this area of the brain of rats in Groups 1 and 2, compared with levels in the same brain region of rats from Group 3. These, a priori, comparisons were tested with Mann-Whitney U statistic, setting a level of significance $< .05$. The analysis, comparing Group 3 with Group 1, gave a value of $U = 4$; $p = .048$ which allowed rejection of the null hypothesis and, consequently, it could be concluded that the levels of noradrenaline in the lower brainstem of rats from Group 3 were higher than levels in rats from Group 1. A comparison of Group 3

with Group 2 (U = 3; p = .028) also confirmed that the levels of noradrenaline in the lower brainstem of the control rats (Group 3) were higher than comparative levels in rats from Group 2. Because of the reservations concerning the data, and despite the fact that only relative levels were important for the suggested hypothesis, a more stringent analysis was performed to test, a posteriori, whether there was sufficient evidence from the data to conclude differences in levels of noradrenaline in the lower brainstems of rats from the three groups of animals. A procedure proposed by Nemenyi (1963) which is based on the Kruskal-Wallis test was used in order to control the comparison-wise error rate at α. The procedure is described in Kirk (1968) and the tests indicated no significant differences between any two of the experimental groups in the levels of noradrenaline of the lower brainstem.

Noradrenaline Content of the Heart.

The levels of noradrenaline in the pairs of bulked hearts of rats in the three experimental groups are presented in Table 8.E. These absolute levels were very low (approximately 20 percent) compared with the levels of cardiac noradrenaline in the rat which have been reported in other studies (Gordon, Spector, Sjoerdsma and Udenfriend , 1966; Bhagat, 1969; Borchard and Vogt, 1970; DiCara and Stone, 1970). It was felt that these low levels may have reflected depletion of noradrenaline owing to the long period of storage. Consequently, fresh hearts from six rats which had been maintained in the Durham colony were immediately assayed in the same manner as the tissue obtained from the experimental animals. The mean level of cardiac noradrenaline from these non-experimental animals was about three times greater than the mean level obtained from rats in

TABLE 8.E The noradrenaline content of the hearts from pairs of rats in the three experimental, treatment groups. Values are expressed as ugm/gm of tissue.

	Group 1	Group 2	Group 3
	0.107	0.235	0.230
	0.198	0.261	0.123
	0.154	0.147	0.215
	0.208	0.242	0.143
	0.117	0.277	0.219
Mean	0.156	0.232	0.186
SE	± .020	± .023	± .022
Median	0.154	0.242	0.215

experimental Group 3, which had also been merely taken from the home-cage and killed. This indicated that the loss of cardiac catecholamine over the storage period may have been in the region of 68 percent. This was a contrast to the apparent absence of any significant loss of noradrenaline from the lower brainstems of rats over the same period of cold storage. This result is interesting, for DiCara and Stone (1970) have shown that a loss of cardiac catecholamine occurred during a shorter period of cold storage, whilst catecholamine levels in the brain showed no depletion when stored under the same conditions. The reasons for these differential effects are as yet obscure.

The reservations concerning the technique and the possible loss of noradrenaline suggest that the results presented in Table 8.E should again be regarded as a qualitative, rather than a quantitative, record.

Because of the absence of strong a priori comparisons between the experimental groups, together with the reservations concerning the data, stringent a posteriori comparisons between the groups were undertaken using the modified procedure of Nemenyi as described by Kirk (1968). From these more stringent comparison tests, no significant differences between any two of the experimental groups were found and, consequently, it was concluded that there were no significant differences in levels of cardiac noradrenaline in rats from the three experimental groups.

DISCUSSION

(i) Adrenal Assays.

The results of the assays of adrenal catecholamines showed that the experimental procedures involved in both curarisation alone and in the baseline conditions used in the studies of operant conditioning of heart-rate changes presented in this thesis, produced depletion in the levels of adrenaline in the adrenal glands, whilst having little effect on the levels of noradrenaline. These effects would suggest that considerable quantities of adrenal adrenaline were released during the experimental treatments of the rats from Groups 1 and 2. Such effects have been previously described to occur in stressful situations and, consequently, the results offer some support for the hypothesis that the baseline experimental conditions of the experiments presented in this thesis are considerably stressful for the rat.

The effect on the two catecholamines is of particular interest, since other studies have also reported differential effects of various emotional schedules on peripheral levels of catecholamines. Mason, Tolsen, Brady, Tolliver and Gilmore (1968) have shown increases in the urinary levels of adrenaline in monkeys, during avoidance sessions, with significantly less effect on the levels of noradrenaline. Earlier experiments by Mason, Mangan, Brady, Conrad and Rioch (1961) and Brady (1967) have similarly examined the levels of catecholamines in the blood of chronically restrained monkeys during various avoidance schedules. Their findings could be summarised as suggesting that the presentation of an anticipated event produced increases in the levels of noradrenaline, but not of adrenaline, in the blood, while a situation which had elements of novelty, ambiguity or unpredictability and in which no clear-cut

response was required, showed increased levels of both noradrenaline and adrenaline. Ferreira, Gollub and Vane (1969) have recorded increased levels of adrenaline, but not noradrenaline, in the blood of primates exposed to shocks and stimuli paired with shocks. Although differential effects on peripheral levels of adrenaline and noradrenaline have been found in several studies in which animals are exposed to stressful or emotional situations, it would appear that the absolute levels of each catecholamine may have been influenced by the differences in experimental parameters or in the methods of their assay, both of which are in evidence in the studies which have been quoted.

It is interesting that several studies, in which human subjects have been used, have also indicated changes in peripheral catecholamine levels during affective or stressful situations; major articles have been presented and reviewed by Elmadjian, Hope and Lamson (1957); Von Euler, Genzeli, Levi and Strŏm (1959); Hoagland (1961); Silverman, Cohen, Shmavonian and Kirshner (1961); Von Euler (1964); Breggin (1964); Kety (1966); and Van-Toller (1970b). These articles indicate that, not only are catecholamine levels altered by affective situations, but also that particular kinds of affect, or stress, have differential effects on the peripheral levels of adrenaline and noradrenaline. More specifically, it is suggested that aggressive reactions which are directed outwards are associated with increased levels of noradrenaline, whilst anxiety is associated with increases in adrenaline. In contrast to the claims for a positive relationship between affective states and catecholamine output, Frankenhaeuser (1969) has reported that she was unable to detect any such simple relation. It remains for future studies to ascertain the specific variables which control the differential release of adrenaline and noradrenaline. It is

clear, however, that procedures for the two groups in the present experiment, which show increases only in the levels of adrenaline, do have elements of novelty, unpredictability and uncertainty which do not require any specific response and which are, evidently, stressful.

(ii) Lower Brainstem Assay

It was hypothesised that the baseline experimental conditions of either curarisation alone or curarisation plus presentations of light and light and shock would be stressful for the rat and, consequently, decreases in levels of noradrenaline in the lower brainstem of these rats were expected. Stringent statistical analysis did not confirm these hypotheses, although the results were encouraging, despite reservations concerning both the depletion of catecholamine levels in cold storage, and the method of their assay. The possible relationship between depletion of peripheral levels of catecholamines and reductions in levels of noradrenaline in the lower brainstem is most encouraging for studies which attempt to demonstrate the relationship between emotional or stressful states and catecholamine metabolism. The only other study which has investigated peripheral and central levels of catecholamines in the curarised animal has recently been published by DiCara and Stone (1970). This experiment, though conceived through a different hypothesis, has considerable relevance and overlap with the study presented in this chapter. DiCara and Stone postulated that the heart-rate obtained in studies of operant conditioning of heart-rate responses in the curarised rat were produced by changes in level of activity in cardiac sympathetic nerves and that such changes would be reflected in the levels of catecholamines in the heart. They also used control groups of animals to demonstrate that, if such changes occurred, they were not a direct

consequence of either the effects of curarisation alone, or the presentations of the reinforcer (positive electrical brain stimulation) and the operant stimulus. In their study, DiCara and Stone also assayed noradrenaline levels in the lower brainstem. The results of their experiment are somewhat amazing and show that the levels of cardiac catecholamines, and the levels of noradrenaline in the brainstem of the rats shaped for decreases in heart-rate, are lower than the levels in similar tissues from yoked control animals which were curarised and which received presentations of stimuli independent of the rat's own heart-rate. Conversely, both the levels of cardiac catecholamines and the levels of noradrenaline in the brainstem of rats shaped for increases in heart-rate were higher than levels in similar tissues from yoked controls. The results offer support for the suggestion of Adolph (1967) of sympathetic dominance of the heart-rate control in the rat. The exciting suggestion of a relationship between sympathetic response, the level of catecholamine in the peripheral tissue, and the level of catecholamine in the region of the brain controlling that response, obviously requires further study and verification.

There are aspects of DiCara and Stone's study which are discrepant with other studies. Bhagat (1969) has shown that the endogenous levels of cardiac catecholamines show little change during increased sympathetic activity, although the turnover of catecholamines is greatly increased. DiCara and Stone did obtain some measure of noradrenaline turnover in the heart by determining the retention of a labelled isotope during the various experimental conditions. This measure indicated that there was considerable depletion in the levels of labelled noradrenaline in the hearts of rats in both the operant trained rats and the yoked controls, suggesting increased sympathetic activity to the heart in these animals. Somewhat paradoxically, however, the depletion was significantly

greatest in rats shaped for decreases in heart-rate. There was a notable absence of any depletion in labelled noradrenaline levels in cardiac tissue from rats which had been curarised but which had received no operant training or presentations of brain stimulation, a fact very surprising in view of their hypothesis and the study by Koelle (1965) which indicated that curare produced considerable increases in sympathetic activity. What is evident from DiCara and Stone's result is the lack of effect of either the yoked control procedures or the curarisation procedures on the endogenous levels of noradrenaline in the brainstem or the heart. Although these effects are in some agreement with the results of the experiment presented in this chapter, they do conflict with the hypothesis postulated in the introductory comment which suggested that reduced levels of noradrenaline in the brainstem may be produced as a result of baseline curarisation and operant training procedures, since these procedures may be stressful to the animal. The apparent, but non-significant, decreases in levels of noradrenaline in the brainstem which were evident as a result of baseline operant procedures in the present study, but not in the study of DiCara and Stone, may have been accentuated by the use of a negative reinforcer. However, the experiment of DiCara and Stone does support the suggestion of considerable increases in sympathetic activity as a result of the baseline conditions involved in studies of operant heart-rate conditioning in the curarised rat.

(iii) Heart Assay.

Both the results presented in this chapter and those of DiCara and Stone indicate no difference in the endogenous levels of catecholamines in the heart as a result of baseline experimental conditions of curarisation and operant training. It is an

interesting point in DiCara and Stone's study that, although they support the suggestion for increased sympathetic activity during baseline experimental conditions, their results show no change of cardiac catecholamines during such conditions. However, the assumed differences in sympathetic activity between groups of rats shaped for increases and decreases in heart-rate responses did produce differences in levels of cardiac catecholamines in these animals.

CHAPTER NINE

SUMMARY AND CONCLUSIONS

The studies presented in this thesis have been performed at the same time as a multiplicity of related experiments in other laboratories. The early initial studies obtained positive evidence for operant control of cardiovascular responses, though not without some reservations. This success, however, posed a variety of questions which could not be answered by a single experiments, or series of experiments. The studies which have been presented in this thesis were derived in a linear manner, each one being evolved from an earlier experiment and, as a consequence, each contributes additional support for the initial preliminary successful experiment on operant conditioning of heart-rate changes in the curarised rat. In the course of these studies, the experimental results have contributed further to our knowledge of autonomic functioning in greater depth than by merely stressing the importance of operant contingencies in cardiovascular responses. Solely for explanatory convenience, the discussion of the contribution of these studies and those from other laboratories, together with theoretical problems which they have created, will be treated in five sections, as follows:

1. The cumulative evidence for the reality of the phenomenon of operant conditioning of cardiovascular responses.
2. Theoretical and practical problems which have arisen from the use of the curarised animal in studies of cardiovascular conditioning.
3. Theoretical implications of studies on operant conditioning of cardiovascular responses.

4. A proposal for the possible direct importance of operant conditioning of cardiovascular responses in psychosomatic medicine.
5. The curarised animal and the functions of the sympathico-medulla system.

1. The cumulative evidence for the reality of the phenomenon of operant conditioning of cardiovascular responses.

The experiments presented in previous chapters have provided considerable direct support for operant conditioning of heart-rate changes under a narrow range of experimental conditions. During the period of this study, a number of other diverse experiments have been performed which also support the same conclusion. The most notable of these, by Miller and his associates, have not only demonstrated operant conditioning of heart-rate changes in the curarised rat, but have also shown that changes in blood pressure may be effected by operant contingencies (DiCara and Miller, 1968b, 1968c; Pappas, DiCara and Miller, 1970). Their studies have demonstrated the specificity of the response changes, such that operant manipulations were shown to effect changes in blood pressure but not in heart-rate (DiCara and Miller, 1968b). Moreover, it was shown that the response change in blood pressure could be located to a limited region of thebody (DiCara and Miller, 1968c). In this sense of response specificity, the most refined experiment by Fields (1970) has indicated that the curarised rat may learn either increases or decreases in the P-R interval of the EKG signal, independently of changes in either the P-P or P-R intervals.

The evidence for operant manipulations of cardiovascular changes is enhanced by these studies because both increases and decreases in the same

response, and similar changes in a different response, can all be produced using the same reinforcer. In other words, the appropriate response is not only reinforced by a stimulus that produces, as the unconditioned response, the same response which is to be learned. The evidence goes further in that more than one reinforcer has been shown to effect similar operant cardiovascular changes.

An experimental study by Slaughter, Hahn and Rinaldi (1970) has negated the criticism that prior operant pretraining in the non-curarised state may be responsible for success in demonstrating operant manipulation of heart-rate changes in the curarised state, when positive electrical brain stimulation is used as the reinforcer, since variable experience with such operant pretraining had no significant effect on later operant heart-rate changes.

The experimental results presented in the studies of Chapter Six of this thesis support the conclusion that the changes in heart-rate produced during operant conditioning are not an indirect effect of the number of presentations of the reinforcer. These latter studies show that the experimental conditions during which operant manipulations of heart-rate changes may be demonstrated are greater than those confirmed by Miller and his associates in his series of experiments in which equal numbers of the reinforcer were received by animals in the increase and decrease group

In hindsight, possibly the more important experiments are those which have indicated that the principles which apply to operant skeletal responses are also evident in operant autonomic responses. Hothersall and Brener (1969) and DiCara (1970) have shown that operant cardiac responses are extinguished when the response is no longer reinforced. DiCara and Miller (1968d) and Hothersall and Brener (1969) have shown retention of operant heart-rate responses over successive training sessions in the curarised state and that there was successive improvement in the performance of the appropriate responses over such training sessions. Unfortunately, the evidence

for discrimination learning of operant cardiovascular response has not been unequivocally positive, although early studies provided some support. There was never any evidence for discrimination of heart-rate changes between reinforced and non-reinforced stimulus conditions in any of the experiments presented for this thesis, although it has been pointed out that the experimental conditions were not optimal for demonstrations of such learning. Other experiments have also produced negative results: DiCara and Miller (1968c) were unable to demonstrate operant discrimination learning of vasomotor responses in curarised mice, and DiCara and Stone (1970) found no significant differences in heart-rate responses between test and blank trials in curarised rats shaped specifically for heart-rate increases. These negative results are a contrast to their earlier studies (Miller and DiCara, 1967; DiCara and Miller, 1968a) in which positive evidence was found for discrimination learning in operant heart-rate responses in the curarised rat. The reason for such discrepancy in the results is obscure but a series of studies on transfer of operant heart-rate changes from non-curarised to curarised state and vice-versa (DiCara and Miller, 1969a, 1969b; DiCara and Weiss, 1969) were also unconvincing in their demonstration of discrimination learning. The fast repetition of heart-beats in the rat makes reinforcement of single events impossible and, consequently, reinforcement is contingent upon an average number of beats. What is reinforced, therefore, can be either changes in single beats, a few beats, or a small change in most of the inter-beat-intervals. The advantage of Black's (1967) study, using dogs with a slower heart-rate, in which each individual inter-beat-interval is involved in setting criterion levels for reinforcement, and in which the results do show a discrimination in heart-rate between reinforced and

non-reinforced stimulus conditions, is clearly evident.

The cumulative evidence for operant manipulations of heart-rate changes would appear to be considerable. Studies are now required which attempt to elaborate those variables which influence the success of shaping specific changes in the relevant cardiovascular responses, and which attempt to assess the extent and longevity of these changes in both the curarised and non-curarised state. One such variable, which studies presented in this thesis have suggested may be important, is the effect of the reinforcer used on the direction of the cardiovascular response; others have yet to be determined.

2. Theoretical and practical problems which have arisen from the use of the curarised animal in studies of cardiovascular conditioning.

The use of the curarised animal to rule out the possibility of mediation of changes in cardiovascular responses by peripheral skeletal activity has, at least in theory, great advantage in that it allows a direct investigation of change in a more pure form in the autonomic response. Some of the studies using curarised animals have involved simple classical conditioning of the cardiovascular response, and it is inferred from these studies that the changes in response so obtained more clearly represent the true phenomenon of classical conditioning. These studies require, a priori, that operant and classical conditioning are separate processes, the latter form only, governing changes in autonomic responses.

There are several specific criticisms which could be aimed at such studies, not the least of which are the positive experimental results demonstrating operant control of cardiovascular responses. The evidence presented in Chapter Four indicated that, in certain species, the dose level of d-tubocurarine required to rule out the

possibility of peripheral skeletal mediation may block, either partially or wholly, autonomic ganglia and, as a consequence, the conditioned cardiovascular responses in these instances may not truly reflect the processes of classical conditioning. Further, the cumulative results of experiments presented in Chapters Five, Seven and Eight would suggest that the effects of curarisation and subsequent artificial ventilation, may themselves alter autonomic functioning considerably, and classical conditioning inferred from autonomic responses superimposed on a high baseline level of sympathetic functioning is unlikely to represent the specific changes which would occur in the non-curarised state.

A preliminary study on classical conditioning of heart-rate changes in the curarised rat presented in Chapter Six showed a variable conditioned cardiac response which, it was suggested, may have been produced by the effect of operant contingencies. As previously pointed out, this result was a contrast to other studies of classical conditioning in the curarised animal in which stable conditioned responses were obtained. Although such responses were assumed to reflect the true phenomenon of classical conditioning of the autonomic response, it is unlikely that the dose level of curare used in those studies was sufficient to completely suppress EMG activity and, consequently, the response changes obtained may have been mediated by skeletal responding. The influence of operant contingencies in studies of classical conditioning need not necessarily result in a variable conditioned response. It has been argued (Kendler and Underwood, 1948; Shearn, 1961), with only limited success, that the effects of drive reduction operate at the offset of the unconditioned stimulus. When a noxious stimulus of long duration is used, and the unconditioned response is in a single, consistent direction, then, if operant contingencies were in effect,

a stable conditioned response in the direction of the unconditioned response could be expected. If, however, onset of the unconditioned stimulus constitutes the reinforcement, as seems more probable (Hilgard and Marquis, 1961), especially if the stimulus is of short duration, then the form of the conditioned response depends on the direction of the heart-rate response at stimulus onset. This, in turn, depends on the response to the conditioned stimulus alone and to the effects of both operant and classical components of cardiac responding, be they separate processes. The effects of operant and classical components on cardiovascular responding, even in the absence of skeletal effects, would seem difficult to predict. Miller (1969) has indicated that his early attempts at demonstrating the effects of operant contingencies on heart-rate responding were unsuccessful because of the large unconditioned responses to the reinforcers he used, and that the operant components, if present, were masked by other components probably due to classical conditioning. These effects pose considerable problems for investigations of classical conditioning, and it remains for future studies to determine the relative importance of operant contingencies in studies of autonomic conditioning. It is evident that operant and classical conditioning are not easily separable processes and it has been suggested (Black and de Toledo, 1969) that no clear understanding of the basic phenomenon of classical conditioning will be obtained by simple study of a single measure of autonomic response functioning.

In addition to these problems, the effects of curarisation, by producing increases in baseline sympathetic functions, provide difficulties for the interpretation of specific changes in cardiac rate during operant training. The increases in such baseline

functions did not appear to reduce the success in shaping increases in heart-rate, although the effect may not have enhanced the ease in shaping decreases in rate. This conclusion would not appear justified, since other studies which have used positive brain stimulation as a reinforcer (Trowill, 1967; Hothersall and Brener, 1969) have found it easier to shape decreases in heart-rate in the curarised rat. The results of the various studies presented in this thesis suggest that specific changes in both sympathetic and parasympathetic functions in the curarised animal may be influenced by operant contingencies but, unfortunately, there has been little evidence to suggest that similar changes could be effected in the non-curarised state. The effects of curarisation on baseline cardiac functions indicate the dangers in assessing the success of operant contingencies, in particular the effects of variables influencing specific response changes, by changes in that baseline level of responding. In this respect, the study of Black (1967) again has particular value in that the effects of the operant contingencies on heart-rate responding were assessed relative to changes in baseline responses. Similar attempts to measure the discrimination in heart-rate changes between reinforced and non-reinforced stimulus conditions in the curarised rat have provided confusing results.

Much of the implied importance of operant contingencies in cardiovascular changes would be lost if it were not possible to demonstrate the effects of such contingencies in the non-curarised state, or if specific changes in direction of the response could not be produced in that state by operant training. These effects are pre-requisite to the suggested practical importance of operant conditioning of cardiovascular responses in the etiology and treatment of certain psychosomatic symptoms. Recent evidence on this problem has been obtained by Miller and his associates, who have investigated

the effects of transfer of operant cardiovascular changes from curarised to non-curarised state and vice-versa. The first two of these studies were published in 1969 (DiCara and Miller, 1969a, 1969b). One demonstrated that heart-rate changes, shaped successfully in rats under curare, transferred to the non-curarised state, and that subsequent operant training in the non-curarised state further increased the appropriate heart-rate changes. The second study was somewhat different in that initial training was performed in the non-curarised state and effect of transfer to the curarised state was the salient feature. In this latter study, initial shaping of decreases in heart-rate was successful, but increases could not be obtained. On transfer to the curarised state, however, not only did rats shaped for decreases show that response but also rats, which had initially shown no change in response, shaped for increases in rate now displayed the appropriate increases. These changes in the non-curarised state were not enhanced by further training. On re-transfer to the non-curarised state, the appropriate changes in rate were again evident and subsequent training again further increased these heart-rate changes. There are certain features of these experiments which have considerable importance. Firstly, they demonstrate that operant contingencies do effect appropriate heart-rate changes in the non-curarised state; moreover, the differential changes in heart-rate between increase and decrease groups were not consistently related in any way to either respiratory rate or skeletal activity. This result would suggest that in studies of operant conditioning of cardiovascular changes in the curarised state, the response changes are not indirectly mediated by conditioning of motor activity within the higher brain areas. Although transfer of operant effects was evident between the two states, the specific

direction of response, or the degree of change in response, was not always maintained on transfer. DiCara and Miller suggest that such effects may be produced either because of generalisation decrement on transfer from one state to the other, and/or because rats appear unable to learn so well operant heart-rate changes in the non-curarised state when, they postulate, there is more 'noise' produced by the confusing effects of changes in heart action and blood vessel tone, caused by skeletal activity. On the basis of the results from the experiments presented in this thesis which demonstrate the influence of curarisation and subsequent artificial ventilation on baseline sympathetic function, it is far from surprising that heart-rate responses do not transfer, in absolute terms, from curarised to non-curarised state. Two other studies have also investigated the effects of transfer between these two states. In 1969, DiCara and Weiss partly replicated the first experiment by DiCara and Miller (1969a), showing transfer of both heart-rate increases or decreases from the curarised to the non-curarised state. However, in addition, they showed that the rats whose heart-rates had been shaped for increases were more poor at a modified shuttle box avoidance task than rats which had been shaped for decreases in rate. The behaviour of the rats whose heart-rate had shown increases was described as being extremely reactive to the shock itself, squealing, turning toward the tail, whilst between shock they remained immobile. In contrast, rats whose heart-rates had been shaped for decreases in the curarised state, showed more inhibited behavioural reactions to the shock during the avoidance task and between shocks; they merely walked about slowly. It is apparent that these two diverse patterns of behaviour were likely to produce the differences which were obtained between the two groups of animals in their probability of

shock avoidance during shuttle-box performance. It would appear from this study that the 'emotional behaviour' of the rat had been modified by the prior operant conditioning of cardiac responses in the curarised state. A difference in reactivity or emotionality between rats shaped for increases and those shaped for decreases in heart-rate had been previously reported by DiCara and Miller (1968d). The behaviour of the rats, following experiments which have been presented in this thesis, could not be described in similar terms. In every case the animal was extremely non-reactive, showing signs of muscular spasms and often merely walking out of the operant conditioning chamber into a holding cage. Unfortunately, the behaviour of each animal on return to the operant chamber, after complete recovery from tubocurarine paralysis, was not ascertained. The experiment of DiCara and Weiss is particularly interesting in that it demonstrates operant control of behaviour which traditionally has been assumed to be established by classical conditioning. However, the experiment should not necessarily be interpreted at face value, in that it seems to equate heart-rate changes and emotional responses. Rather, it may be that it is some central 'emotional' state which has been manipulated by the operant contingencies, and it is this state which elicits the changes in heart-rate. Thus, there is the possibility of a further hypothetical central state which may mediate the changes in heart-rate shaped by operant contingencies in the curarised state.

The most recent study on the effects of transfer from non-curarised to curarised state adds even more caution to a simple interpretation of the results of operant conditioning of cardiovascular changes. Pappas, DiCara and Miller (1970) found that successful operant conditioning of increases or decreases on blood pressure in the rat did not transfer to the curarised state, although subsequent training

in that state did produce the appropriate responses. It is clear that more studies are urgently required on operant conditioning of cardiovascular responses in the non-curarised state and the subsequent effects of transfer. The experiments by Miller and associates would imply that operant conditioning of cardiovascular responses is more difficult in the non-curarised state, possibly because of the peripheral skeletal effects which produce cardiovascular changes that overwhelm any possible effects of operant contingencies. It may be that the single sessions which have been used in their studies in the non-curarised state have been too short, and that more sessions are required to learn and establish stable operant cardiovascular responses.

There appears to be two considerable disadvantages for studies on operant conditioning of cardiovascular responses which use curarised or non-curarised rats. Firstly, the rapid heart-rate of the rat does not allow direct reinforcement of single events and it is necessary to ascertain the success of operant control by changes in baseline rate. Since such changes would appear to be influenced by curarisation procedures and, subsequently, making it difficult to predict changes on transfer to the non-curarised state, it would be desirable to use an alternative species with a slower heart-rate in future studies. Secondly, there is some difficulty in shaping heart-rate changes in the non-curarised state in the rat, partially because of the schedules it is necessary to use, and such problems do not provide encouragement for further studies on operant conditioning of cardiovascular responses in that state, or for the possible simulation of chronic resting levels of cardiovascular functions in the rat.

3. Theoretical implications of studies of operant conditioning of cardiovascular responses.

The positive evidence for operant conditioning of cardiovascular responses poses a clear problem for Mowrer's (1947) two-process learning theory. More specifically, it seriously questions the multiplicity of studies on autonomic conditioning which have been based on the premise of that theory; that is, autonomic responses are subject in learning only to the laws of classical conditioning. As yet, the demonstrations of operant autonomic conditioning have been confined to a relatively limited set of experimental conditions in order to satisfy the theoretical constraints related to the problem of indirect mediation of the autonomic response. Although the conclusions from these experimental findings embarrass those theorists who have stressed the separation of classical and operant conditioning, primarily on the grounds that the type of response involved in each was supposed to be typically different, the evidence from the experiment on operant conditioning of heart-rate changes in the functionally decorticate, curarised rat does offer tentative support for a separation of these forms of learning. This latter study gave additional support for the suggestion that operant conditioning depends on the functional integrity of the cerebral cortex (Steele-Russell, 1966). The support for this hypothesis appeared particularly impressive in that the response involved, heart-rate, was assumed to be controlled subcortically. However, this assumption has not yet been substantiated, nor can it be, until the problem of mediation has been resolved. It has yet to be convincingly demonstrated that operant cardiovascular responses are not an indirect consequence of operant conditioning of activity in higher brain centres controlling motor activity. An alternative possibility

which is equally damaging to Steele-Russell's hypothesis, is that the heart-rate changes are produced by operant conditioning of activity within central, cerebral cardiovascular areas which, it has been suggested, may involve the same mechanisms in the central nervous system as those for skeletal responses. Positive evidence for such common central mediating mechanisms linking cardiac and skeletal events has been provided by a number of neurophysiological experiments (Rushmer and Smith, 1959; Rushmer, 1961). For example, Rushmer and Smith (1959) have shown that virtually all portions of the central nervous system which consistently yield cardiovascular responses when stimulated, also induce behavioural changes which would normally be associated with such cardiovascular adjustment. It is partially on this evidence that Obrist, Webb, Sutterer and Howard (1970) have argued that attempts to condition cardiovascular responses by operant contingencies have ignored the logical, biological adaptiveness of the difficulty in separating cardiovascular functions from skeletal or somatic functions. This, they argue, is the same error that Mowrer made in postulating his original separation of autonomic and skeletal responses. The point of Obrist et al. is acute, and it has been ignored in a considerable number of studies of autonomic responses performed by psychologists; however, his comments in this specific instance are not justified. In an early article, DiCara and Miller (1968a) show themselves to be acutely aware of the close relationship between central skeletal and cardiac control mechanisms, although they strongly argue that operant cardiac components are independent of central skeletal mechanisms. Black (1967) also has shown that he is aware of the same problem and, although his study demonstrated that operant conditioned heart-rate changes in the dog are closely related to changes in EMG activity, he has presented additional evidence which

suggested that operant cardiac changes can be shown to occur in the absence of concomitant changes in EMG activity. Nevertheless, it is clear that Black, unlike Miller and associates, does concur with the views of Obrist et al, for his data logically supports his conclusion that, although operant heart-rate responses are not necessarily associated with skeletal movement, they are associated with central processes involving initiation and maintenance of movement. Those studies which have demonstrated that operant conditioned heart-rate changes successfully shaped in the curarised state transfer to the non-curarised state, and that they are not consistently related to either skeletal activity or respiration rate in that state, clearly support the position of Miller and associates.

There is no doubt that the problem of mediation is important, however, it is essential to realise that the experiments generated by Mowrer's theory and the criticisms concerning those experiments by Obrist et al, are at two different levels. The original theory of Mowrer, and the experiments generated by it, were concerned with overt and covert peripheral responses mediated by the autonomic and somatic nervous systems, whilst the problem of mediation has now largely focused on central processes. The experimental study of mediation at the level of central processes is extremely difficult and the postulated relationship between central somatic and cardiac processes does not preclude other central states, such as a postulated 'emotional central state', affecting changes in cardiac responses. In order to check these possibilities, one must be able to differentiate amongst the various possible mediating central processes and processes that directly control the cardiac responses. It would seem potentially more profitable to analyse and assess the importance of variables effecting operant changes in cardiovascular responses. However, there

are other reasons than those of practical convenience for diverting attention away from the problem of mediation. Mowrer clearly postulated the response separation of operant and classical contingencies at the peripheral level; if, however, it had been considered at the level of central processes, and if mediation of the operant cardiac responses by alternative central processes were to be demonstrated, then one could maintain a theoretical position that these cardiac responses could be directly conditioned by classical procedures. Nevertheless, whether this mediation is responsible for the results of the heart-rate changes in these studies of operant conditioning of cardiac changes in the curarised rat or whether the results are a consequence of direct operant conditioning of such changes, the power of Mowrer's theory is considerably reduced. The changes in cardiac responses could be produced by classical conditioning, operant conditioning, or by the operant conditioning of some mediating response. Hence, the differentiation between operant and classical conditioning could not be related in any simple way to the autonomic/skeletal response distinction.

4. A proposal for the possible direct importance of operant conditioning of cardiovascular responses in psychosomatic medicine.

In a number of articles, Miller and associates have suggested that abnormal resting levels of autonomic functioning may be produced by operant learning (Miller, 1967, 1969; DiCara, 1970; Miller, DiCara, Solomon, Weiss and Dworkin, 1970). Since it would appear that visceral responses are subject to instrumental learning, then reinforcement of responses should not be limited to unconditioned

stimuli that elicit the specific change which is to be learned, but the response should be modified by a great variety of rewards and punishments. As a consequence, it is clearly possible to learn visceral responses such as hypertension, which are involved in psychosomatic symptoms. This indicates a means of therapy for essential hypertension based upon conventional learning principles in an analogous manner to the procedures used in attempts to modify other types of abnormal behaviour (Wolpe, 1958; Mowrer, 1953, Yates, 1971). There is substantial evidence to support the importance of operant contingencies in the control of cardiovascular responses in humans. Hnatiow and Lang (1965) and Sroufe (1969) have shown that subjects are able to learn to stabilize their own cardiac rates. Other studies have demonstrated that either increases or decreases in heart-rate, or blood pressure, of humans can be brought under operant control by the use of positive or negative reinforcers (Brener, 1966; Engel and Hansen, 1966; Engel and Chism, 1967; Snyder and Noble, 1968; Plumlee, 1969; Broome, 1969; Brener and Kleinman, 1970; Shapiro, Turksky and Schwartz, 1970a, 1970b). Unfortunately, the controls for possible mediation of the cardiovascular responses in these studies have rarely been adequate. In theoretical terms, therefore, these studies provide less substantial evidence for the operant control of cardio-vascular responses than studies in which curarised animals have been used. However, in relation to the suggested importance of operant contingencies in essential hypertension the problem of indirect mediation of the cardiovascular response is somewhat a pseudoproblem. Indeed, Von Eiff (1970) has shown that increases in muscle tone are associated with essential hypertension. Again, research would be more profitable if it focused on determining the extent to which operant contingencies can effect changes in the cardiovascular responses of humans, and

the variables which control such changes. It has been suggested, and experimentally verified, that the degree of external feedback of the response changes affects the success of demonstrating operant conditioning of cardiovascular responses in humans (Lang, Sroufe and Hastings, 1967; Brener, Kleinman and Goesling, 1969). Experiments with curarised rats have indicated the importance of the unconditioned response to the reinforcer for the success in shaping specific changes in cardiac rate in a given direction. This would imply that the range of reinforcers which influence operant responses in a specific direction may be considerably less than would be expected on the almost completely transitional mode of operation of reinforcers in operant conditioning of skeletal responses. It would be essential in operant therapeutic procedures for hypertension to choose a reinforcer which did not produce, as the unconditioned response, consistent blood pressure increases acting in the opposite direction to the response required. A similar mistake has often been found in attempts to modify skeletal responses by operant techniques. Gwinn (1949) demonstrated that shock applied to the feet of rats was ineffective in punishing the running response, the unconditioned effects of the shock being incompatible with remaining immobile.

Though exciting in conception, these suggestions for the practical importance of operant contingencies in cardiovascular functions are still speculative. The etiology and pathogenesis of essential hypertension remains unknown, despite a considerable degree of investigation at both physiological and psychological levels (Buss, 1961; Korner, 1970, Von Eiff, 1970, Grollman, 1971). The possibility of the importance of operant contingencies is attractive because of the familiarity of the concepts involved. However, this suggestion is only one of a multiplicity of alternatives which have been offered for the development of essential hypertension

and, though easily reconciled with psychological theories which stress the importance of environmental influences in bridging the gap between temporary rises in blood pressure and chronic elevation of blood pressure (Buss, 1961), it is a considerable jump from a demonstration of the effects of operant contingencies in cardiovascular functions to determining that such principles account for the development of essential hypertension. Considerable further research is required into both the physiological mechanisms responsible for essential hypertension and the environmental influences effecting its development. Nevertheless, the success in demonstrating operant conditioning of cardiovascular responses gives encouragement for further investigation of the possibilities for reducing chronic levels of cardiovascular responses by methods based by operant principles at both the clinical and experimental levels.

5. The curarised animal and the sympathico-medulla system.

The specific changes within the autonomic nervous system responsible for heart-rate changes during studies of classical conditioning have received particular attention (Obrist, Wood and Perez-Reyes, 1965; Hastings and Obrist, 1967). However, with the exception of this thesis and the experiment of DiCara and Stone (1970), the studies on operant conditioning of heart-rate changes have merely speculated as to the mechanisms responsible for the specific changes obtained. The lack of success in shaping operant increases in heart-rate in immunosympathectomised or adrenal demedullated rats indicated that both the sympathetic cardio-accelerator nerves and the adrenal medulla are required to maintain increases in the baseline heart-rate response in the curarised rat. As a consequence, it was hypothesised that the baseline conditions involved in studies

of operant heart-rate conditioning in the curarised animal were stressful and involved increased levels of sympathetic functioning. A subsequent experiment provided some positive evidence for this hypothesis in that a significant depletion was found in the levels of adrenaline in the adrenal glands of rats which had been subjected to the baseline conditions of curarisation and artificial ventilation. The effects of these physiological manipulations were summarised and interpreted as suggesting that both the sympathetic nervous system and the adrenal medulla contributed to the range of control of heart-rate increases. A parallel hypothesis of an 'adaptive range of response' was postulated by Ramey and Goldstein (1957) to explain the effects of sympathectomy and adrenalectomy on physiological functions in stressful situations. Their concept was, however, somewhat different, in that it was the functions of the adrenal cortex which were emphasised for the maintenance of the receptivity of target organs for the influence of sympathetic catecholamines. The compensatory systems and the sympathetic dominance of the rat would suggest that a reduced range of response is, however, only found in experimental situations which require a high baseline level of sympathetic function, i.e. which are stressful. This interpretation, applied to the results of the present studies, is particularly impressive both because the response involved, heart-rate, is known to be directly affected by changes in sympathetic and adrenal functions, and because the response has been treated and manipulated in a behavioural, rather than a physiological, context.

Despite the simplicity of this concept of a reduced range of response within a stressful situation, the wide influence of Mowrer's (1947) version of two-process learning theory with its stress on classical conditioned autonomic responses acting as mediators of

instrumental responding, implied that any manipulation of the sympathetic nervous system would alter the efficiency of instrumental responding. The results of such early studies (Auld, 1951; Wynne and Solomon, 1955) were inconclusive because of internal inadequacies within the experiments (see Van-Toller, 1970a). Later experiments have investigated the effects of both immunosympathectomy (Wenzel, 1968; Van-Toller, 1970a) and adrenalectomy or adrenal demedullation (Moyer and Bunnell, 1959; Levine and Soliday, 1962, Appleby, 1964) on instrumental behaviour and the results, though varied have been inconclusive. Even assuming that instrumental behaviour is mediated by autonomic responses as postulated by two-process theories, such studies are based on an inadequate understanding of the functions of the sympathico-medulla system. A series of unpublished studies by Van-Toller (1971) have demonstrated consistent decrements in skeletal instrumental behaviour in immunosympathectomised mice only when the animals are under considerable physiological stress (that is, when their 'sympathetic system' is functioning at near asymptotic levels). The results of the present series of experiments would support these conclusions for the effects of sympathetic functioning.

There was no deficit in either the immunosympathectomised or demedullated rats in the brief tachycardia responses to stimuli during the operant training schedules, but only in maintaining the increases in baseline levels of response required by the specific schedule within, what appears to be, a stressful situation.

APPENDIX

The Trihydroxyindole Methods of Catecholamine Estimation Using Potassium Ferricyanide as the Oxidant.

The method reported below was shown to the author by Dr. C. Van-Toller. The basic principles of fluorescence assay of catecholamines were gained from Udenfriend (1962). Crout (1961) has presented a lucid account of the trihydroxyindole differential pH method, together with a worked example.

1. Adrenal Assays.

Dissected adrenals were stored $^{0}/_{N}$ at $-15^{o}C$ and the following day ground up in an iced Potter's glass homogenizer with 4 mls. of iced 0.01N hydrochloric acid. The homogenate was accurately made up to 25 ml. in a volumetric flask using the dilute acid, and stored in a refrigerator until the actual assay procedure was carried out later in that day. Standard solutions of adrenaline and noradrenaline were made up from stock solutions of adrenaline bitartrate or noradrenaline bitartrate containing 1mg/ml in 0.1N hydrochloric acid which had been made up the day previous to the first assay and then stored at $2^{o}C$. These stock solutions were used on subsequent assays when standard solutions were required.

The assay technique used was a trihydroxyindole, differential pH reaction with potassium ferricyanide as the oxidant. This technique uses the fact that both monoamines are oxidised to the aminochrome stage at pH 6.0 whereas only adrenaline undergoes any significant oxidation at pH 3.5. Prior to the assay, the diluted homogenates were shaken and spun down for 5 minutes. Two mls. of the supernatants were pipetted into each of two tubes, one containing 2 mls. of acetate buffer pH 3.5, the other containing 2 mls. of acetate buffer pH 6.0.

The reading tubes contained the following solutions:

pH 3.5	pH 6.0
2 mls sample	2 mls sample
2 mls pH 3.5	2 mls pH 6.0 buffer
0.1 ml 0.5% zinc sulphate	-
0.1 ml 0.75% potassium ferricyanide	0.1 ml 0.25% potassium ferricyanide
leave 3 minutes	
2 mls alkaline ascorbic acid	2 mls alkaline ascorbic acid
read after 12 minutes	

After the addition of each solution the tubes were mixed using a mechanical shaker. The standard solutions of adrenaline and noradrenaline were also set up at pH 3.5 and pH 6.0. The 'blank' tube contained 2 mls of the 0.01N hydrochloric acid, used to dilute the homogenate and the 0.25% potassium ferricyanide was added after addition of the alkaline ascorbic acid. The relative fluorescence obtained from the sample was read from an Aminco-Bowman Spectrophotofluorometer. Optimal readings of noradrenaline levels were determined by excitation of the sample with wavelength of light of 395 millimicrons and peak fluorescence was subsequently found at 505 millimicrons. Equivalent values for optimum readings of levels of adrenaline are: excitation, 410 millimicrons and fluorescence, 520 millimicrons.

2. Assays of the Heats and Lower Brainstems.

These tissues were assayed only for the presence of noradrenaline and used a trihydroxyindole reaction at pH 6.5 (levels of adrenaline in tissues other than the adrenals is negligible). After dissection each type of tissue was bulked at random into two organs from a similar treatment group and stored at -15°C until assayed on a later occasion. The tissues were ground up using a pestle and mortar with a little sand added. After grinding, the tissue and sand was then mixed and transferred into a small centrifuge tube using a total of 9 ml of 0.4N perchloric acid in three washings. The tubes were then sealed and stored at -15°C $^{O}/_{N}$. The following day the tubes were thawed, mixed and centrifuged for 5 minutes. The supernatants were decanted into a large clean centrifuge tube and the pellets resuspended with 1 ml of 0.4N perchloric acid. Following a further centrifuging for 5 minutes the washings were added to the original supernatant (during this and the following process the tubes were immersed in ice). The pH of the supernatants were then adjusted to pH 6.0 using a pH meter, a magnetic stirrer and acid and alkaline solutions (solutions of 4N and 0.4N potassium hydroxide and 1N and 0.1N hydrochloric acid were used to make the pH adjustment). The pH adjusted solutions were then left to stand for half an hour before being centrifuged for 5 minutes, to remove the potassium perchloride precipitate that had formed. The supernatants were then run onto ZEO-KARB resin columns. The resin columns were washed with 20 mls of iced distilled water and finally the noradrenaline was eluted using 10 mls of iced 1N hydrochloric acid. The eluates were collected in small bottles, capped and stored at 2°C until all the samples had been collected. Two 10 ml volumes of noradrenaline containing 1 µg/ml in 0.01N hydrochloric acid were also run through the columns to serve as internal standards.

The glass columns consisted of a stem, narrowed at the bottom, 13.5 cm long having an internal diameter of 0.6 cm attached to an upper glass reservoir which was 9 cm high and had an internal diameter of 3.1 cm. The resin columns were prepared by placing a plug of glass wool at the bottom of the stem and placing a resin column of approximately 2.0 cm on top of the plug. The assay tubes were prepared as follows:

1.5 ml sample)
) pH of mixture to be
1.5 ml pH 9.9 buffer) 6.5; this was carefully checked.

0.1 ml 0.25% potassium ferricyanide

leave for 3 minutes

2.5 ml alkaline ascorbic acid

read after 25 minutes

After the addition of each solution, the tubes were mixed using a mechanical shaker. The 'blank' tube contained 15mls of the 0.1N hydrochloric acid, and the 0.25% potassium ferricyanide was added after the addition of the alkaline ascorbic acid. The relative fluorescence obtained from the sample was read from an Aminco-Bowman Spectrophotofluorometer. Peak wavelength for excitation of the sample was again 395 millimicrons, and peak fluorescence was obtained at 505 millimicrons.

3. Solutions for Catecholamine Assays.

Sand:

The sand was washed several times with deionized water and finally with a 1% solution of ethylenediaminetetra-acetate (EDTA).

Preparation for the resin for the tissue assay:

Approximately 200g of resin (Zeo-karb 225 (SRD16): 8% cross linked over 200 mesh) were suspended in deionised water and the cloudy supernant was decanted after approximately 10 minutes. This decantation process was carried out until the supernant remained clear after 10 minutes. The resin was then transferred to a large glass column fitted with a sintered glass filter disc and a stop-cock.

The column was washed with the following solutions:

(a) 2.5 litres 2N sodium hydroxide containing 1% EDTA.

(b) 1 litre deionised water (to remove the alkali).

(c) 2.5 litres 2N hydrochloric acid.

(d) 1 litre deionised water (to remove the acid).

(e) 4-5 litres of 0.1M phosphate buffer (pH 6.5) containing 0.1% EDTA.

The phosphate solution was run through until the pH of the column effluent was 6.5 (the resin could be seen in the golden-yellow form). The resin was removed from the column, washed several times with deionised water and stored ready for use.

Alkaline ascorbic acid solution:

This solution consisted of 1ml of a 2% $^{W}/_{V}$ ascorbic acid and 9mls of 5N sodium hydroxide to which 0.2ml of a 97% ethylenediamine (EDA) solution was added. The alkaline ascorbic acid solution is oxidised very quickly and it is important to make up the volume required prior to using it.

Acetate buffers for adrenal assay:

Acetate buffer pH 3.5: to 47mls of 1M acetic acid was added 3mls of 1M sodium acetate ($CH_3COONa .3H_2O$; mol. wht. 136.08).

Acetate buffer pH 6.0: to 47 volumes of 1M sodium acetate (hydrated, see above) was added 3 volumes of 1M acetic acid.

pH 9.75 - 9.90 buffer for tissue assays:

To 205 mls of 2M acetic acid pH 5.0 was added 45mls of 5M Potassium carbonate (anhydrous).

Acetate buffer pH 5.0; To 2 volumes of 2M sodium acetate (hydrated) was added 1 volume of 2M acetic acid.

Phosphate buffer, pH 6.5, for ZEO-KARB resin column:

Stock solutions: (a) 0.2M monobasic sodium phosphate.

(b) 0.2M dibasic sodium phosphate.

To 342.5ml of solution (a) was added 157.5ml of solution (b) and diluted to 1 litre. 0.1% EDTA was then added.

Stock solution of noradrenaline:

99.68mgs of noradrenaline bitartrate ($C_8 H_{11} NO_3 H_6 O_6$, H_2O; mol. wht. 337.29) was dissolved in 0.1N hydrochloric acid and made up to 100mls in a volumetric flask using the dilute acid solution.

Stock solution of adrenaline:

181.92mgs of adrenaline bitartrate ($C_9 H_{13} NO_3 .C_4 H_6 O_6$; mol. wht. 333.30) was dissolved in 0.1N hydrochloric acid and made up to 100mls in a volumetric flask using the dilute acid solution.

Standard solutions were prepared by making dilutions in 0.01N hydrochloric acid prior to any assay.

All glassware was immersed overnight in a solution of Decon (British Hydrological Corporation) and rinsed thoroughly in tap

water on the following day. Deionised, distilled water was used as a final rinse for all glassware and also to make up any chemical solutions.

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