HEART-RATE CONDITIONING IN RATS USING VAGAL NERVE STIMULATION AS THE UNCONDITIONED STIMULUS

By

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TABLE OF CONTENTS

Acknowledgements i

List of Figures ii

Introduction 1

Experiment I 13

Methods 14

Results. 20

Experiment II 31

Results. 32

Experiment III 35

Results. 36

Discussion 39

Summary and Conclusions. 44

References 46

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LIST OF FIGURES

1. Mean pretest CS-alone and acquisition difference scores of the vagal experimental, vagal control, chest-shock experimental, and chest-shock control groups averaged over blocks of four trials.

2. Mean acquisition difference scores of the vagal experimental, vagal control, chest-shock experimental, and chest-shock control groups for each 2-sec. period during the CS-UCS interval.

3. Mean pre-CS HR of the vagal experimental, vagal control, chest-shock experimental, and chest-shock control groups averaged over four trial blocks during acquisition.

4. Sample acquisition trial of a vagal experimental subject showing the UCR to vagal stimulation.

5. The mean HR UCRs of the vagal experimental and control groups as a function of blocks of time after the onset of the UCS and the mean HR UCRs of the chest-shock experimental and control groups as a function of blocks of time after the termination of the UCS.

6. Mean pretest CS-alone difference scores for Day 1 and mean acquisition difference scores for Day 1 and Day 2 of the vagal experimental group averaged over four trial blocks.

7. Mean acquisition difference scores of the neck-stimulation experimental and control groups averaged over blocks of four trials.

8. Mean HR UCRs of the neck-stimulation experimental and control groups as a function of blocks of time after the termination of the UCS.

INTRODUCTION

A major issue in the study of classical conditioning is whether the unconditioned stimulus (UCS) must activate some motivational process or if simple contiguity of the conditioned stimulus (CS) with the UCS is sufficient for conditioning to occur (Kimble, 1961; Osgood, 1953; Spence, 1951). In appetitive salivary classical conditioning, food deprivation provides the primary source of motivation. The UCS, food, elicits the salivary unconditioned response (UCR) that is paired with the CS. If the subject is not food deprived, the food elicits only a small UCR and conditioning is impaired. In aversive classical conditioning, the source of motivation is not as readily specified as in the appetitive case. An UCS such as electric shock is typically employed to elicit the UCR. In addition to the UCR, the shock elicits pain, fear, and other emotional responses that are assumed to provide the source of motivation. Another more subtle form of motivation in appetitive and aversive classical conditioning may be the presence of instrumental contingencies. In this instance the conditioned response (CR) may be reinforced by its ability to instrumentally modify the consequences of the UCS.

One of the methods employed to examine the role of motivation in classical conditioning has involved the use of electrical stimulation of the motor cortex as the UCS. Motor cortex stimulation typically elicits a stereotyped mechanical movement that from all appearances is completely devoid of any noxious or motivational properties.

Apparently, the earliest published experiment using motor cortex stimulation as the UCS in classical conditioning was performed by Loucks (1935). He paired an auditory CS with electrical stimulation of the sigmoid gyrus of dogs that were suspended in a hammock. Depending upon the location of the electrode, the UCS elicited a relatively discrete forelimb or hind-limb flexion. After numerous CS-UCS pairings none of his dogs developed conditioned movements to the CS. However, when food was used to reinforce the limb flexion, conditioning rapidly occurred. Loucks noted that cortical stimulation did not produce any reactions in his animals that could be classed as noxious or painful. From these experiments, he concluded that mere repetition or contiguity of the CS and UCS was not sufficient for conditioning to occur.

Following Louck's original study, several investigators have been successful in conditioning motor movements using cortical stimulation as the UCS. These studies have been summarized and extended further in a recent paper by Doty and Giurgea (1961). Doty and Giurgea used electrical stimulation of the motor cortex as the UCS and a tone or stimulation of another cortical point as the CS. Four dogs, two monkeys, and two cats served as subjects in the conditioning phase of the experiment. The dogs were loosely restrained in a standing position by placing their legs through plastic loops. The cats were immobilized by placing their heads in a plastic stock, while the monkeys were kept in standard primate chairs. Those subjects that showed successful conditioning developed a CR in approximately 30 to 70 trials. Two subjects failed to condition using motor cortex stimulation. However, both of these subjects

were seizure prone and did not readily condition using avoidance and classical conditioning procedures employing electric shock as the UCS.

In an attempt to access the motivational properties of motor cortex stimulation, the four dogs were trained to lever press for food. Once this response was established, the animals then received motor cortex stimulation each time they pressed the lever. The assumption was that any aversive or motivational properties of the UCS would be revealed by a decrease in the rate of pressing. In two of the subjects, it appeared that the cortical UCS was aversive. Apparently during the five months since electrode implantation, meningeal trigeminal fibers had grown into one electrode placement, while the other electrode was located in a brain region (field H_1 of Forel) that appeared to be aversive when stimulated. The observations in these two subjects that the cortical UCS was aversive were confirmed in that stimulation abruptly caused cessation of lever pressing. However, when motor cortex stimulation had given no previous indication of being aversive, presentation of the cortical stimulation during lever pressing did not disrupt the pressing response except for the momentary hesitation caused by the forceful skeletal reactions. On the basis of the latter findings, Doty concluded that motor cortex stimulation, which was used in the classical conditioning phase of the study, did not involve the activation of some motivational process.

Wagner, Thomas, and Norton (1967), using motor cortex stimulation as the UCS in three dogs, replicated and extended the work of Doty and Giurgea. The training procedures which were used included normal conditioning, discrimination, differentiation, and a threshold test. During

normal conditioning a tone was paired with the UCS. In the discrimination procedure, one CS (CS+) was always paired with the UCS, while the other CS (CS-) was presented alone. The differentiation procedure consisted of pairing one CS with a forelimb UCR, while another CS was paired with a hind-limb UCR. All three conditioning procedures were successful in obtaining reliable conditioning. One subject given normal conditioning failed to show any CRs in over 2,000 trials. It is important to note that contrary to the other subjects, this animal was allowed to adopt a sitting posture during the conditioning operations.

In the second part of the study, a threshold UCS (UCS_t) was determined that would elicit a UCR 50% of the time. Presentation of the CS+ from the previous discrimination training with the UCS_t reliably increased the number of UCRs above the number obtained by pairing the CS- with the UCS_t. According to Wagner et al., this outcome provided evidence for a form of learning that can be demonstrated without relying on the occurrence of an overt CR.

The overall variability of the CRs and the failure of the one subject allowed to adopt a sitting posture to condition led Wagner et al. to suggest that instrumental contingencies may have been operating during the classical conditioning phase of the study. Thus, the CR may have been reinforced through its ability to reduce the abruptness or forcefulness of the UCR. According to this view, the subjects chose to make a CR-modified UCR rather than a UCR. This interpretation did not imply that the motor cortex stimulation was noxious or aversive.

In a second study, two dogs were trained to press a panel for food reward. Later, the delivery of cortical stimulation was made contingent upon the panel press. The dogs were given a choice between two panels. On one panel the UCS was signalled by a CS and on the other panel an unsignalled UCS was presented. Since the dogs consistently chose the panel with the signalled UCS, Wagner et al. concluded that instrumental contingencies in the form of preparatory responses may have been operating in the establishment of the CRs to cortical stimulation. Nevertheless, the threshold technique still indicated that some conditioning occurred in the absence of any CR which could modify the UCR.

The role of posture in the development of a CR to cortical stimulation was examined further in a study by Thomas (1971). Using essentially the same conditioning procedures employed by Wagner et al. (1967), Thomas varied the dog's posture during conditioning. He reasoned that if the development of a CR is related to preparatory postural adjustments in anticipation of the UCR, then the larger the effect of the UCR on the subject's equilibrium, the greater the utility of a postural adjustment. From this it follows that the larger the shift in equilibrium caused by the UCR, the higher the probability of a preparatory CR.

Thus, a wide posture group (legs held widely apart) would experience greater shifts in equilibrium as a result of the UCR and consequently show more CRs than a corresponding narrow posture group (legs held closely together). Thomas' speculations were confirmed in that the number of overt CRs in the wide posture group was reliably greater than in the narrow posture group.

These results clearly indicated that posture can have a sizeable effect on anticipatory CRs, and that in order to evaluate whether

conditioning can take place in the absence of motivation, response-produced sources of motivation must be carefully controlled. Although none of the studies using cortical stimulation as the UCS provide a crucial test to answer the question of the necessity of motivation in conditioning, taken as a whole, they seem to indicate that if motivational properties are required, they are very minimal and perhaps exist only in the form of instrumental contingencies.

A number of studies involving the autonomic nervous system have been reported which bear on the question of contiguity in classical conditioning. These studies employed UCSs that involve varying degrees of central nervous system (CNS) activation.

Teitelbaum, Gantt, and Stone (1956) attempted to obtain cardiac CRs to injections of acetylcholine which caused cardiac inhibition followed by acceleration as the UCR. The dogs were given 200 repetitions of an auditory CS paired with an intravenous injection of acetylcholine. The results indicated that no conditioning occurred in any of the subjects. However, rapid cardiac conditioning was obtained in the same subjects if a painful stimulus, i.e., electric shock, was substituted for the acetylcholine injections.

Gerall, Sampson, and Boslov (1958) reported negative findings in an attempt to condition pupillary dilation in humans using a decrease in illumination as the UCS. Groups that received shock as the UCS developed reliable conditioned pupillary responses.

Young (1958) attempted to obtain conditioned pupillary constriction in humans using a change in convergence or accommodation as the UCS. No conditioned pupillary changes were observed during the 150 conditioning

trials. He was unable to replicate the Gerall et al. findings in that no evidence of pupillary conditioning was obtained using an electric shock UCS.

Attempts to obtain salivary conditioning or conditioned gastric secretions using pilocarpine and histamine respectively have been unsuccessful. Since the action of these drugs is directly on the end organ, it appeared that entirely peripherally acting UCSs were not adequate for conditioning (Finch, 1938; Katzenbogen, Loucks, & Gantt, 1939). However, using food or morphine, both known to elicit emotional reactions, salivary and gastric responses were readily conditioned.

Perez-Cruet, Jude, and Gantt (1966) attempted to condition extrasystoles by electrically stimulating the myocardium. This UCS induces extrasystoles that are not mediated through CNS influences on the heart, but are a result of direct stimulation of the heart muscle. None of the dogs in this study developed cardiac CRs after receiving 100 or more reinforced trials. These results confirmed their belief that any UCS whose action is solely without CNS involvement cannot be used to establish a classically conditioned response.

Most recently, Sideroff, Schneiderman, and Powell (1971) investigated the motivational properties of septal stimulation as the UCS in classical heart-rate (HR) conditioning in rabbits. Using three UCS intensities, they found that conditioning failed to occur in the lowest intensity group, even though reliable HR UCRs were elicited. Conditioned HR decreases were observed in the medium and high intensity UCS groups.

Later, using a shuttlebox apparatus, Sideroff et al. demonstrated that approach responses were made to the low intensity UCS and escape

responses to the two highest intensities. In a self-stimulation experiment in which all the animals chose to press for septal stimulation, the rate of pressing decreased as UCS intensity increased. At the two highest intensities the subjects pressed the bar and then exhibited fear or escape responses. This study indicated that a UCS which produced a reliable HR UCR need not lead to conditioning. It also appeared that successful conditioning was related to aversive properties of the UCS.

Only one study has been reported employing direct stimulation of the vagus nerve as a UCS (Andrus, Gantt, Plumlee, & Gross, 1969). In this study, the vagus nerve in 16 dogs was exposed and severed below the larynx. A 12-sec. tone was reinforced for the last 6 sec. with stimulation of the cephalad portion of the nerve trunk. Vagal nerve stimulation elicited a somewhat variable UCR which consisted of several components. Stimulation of the cephalad portion of either vagi provoked deep, frequently accelerated, respiratory movements with forceful expiration, sometimes followed by apnea lasting up to 15 sec. In the majority of animals, stimulation of the left vagus resulted in transient tachycardia during hyperventilation, changing to bradycardia during the interval of apnea. Stimulation of the right vagus caused increased HR during hyperventilation that remained increased for an interval after the hyperventilation ceased. In either case, systolic and diastolic blood pressure fell by 20-60 mm Hg. Since only the central stump was stimulated (ignoring antidromic stimulation), the above changes can be attributed largely to reflexes initiated by stimulation of afferent fibers from tension receptors in the esophagus, tracheobronchial and pulmonary stretch receptors, pulmonary and systemic arterial baroreceptors, atrial receptors,

and ventrical pressure receptors. These investigators reported that after several hundred conditioning trials no conditioned respiratory, blood pressure, or HR changes were observed.

Andrus et al. (1969) proposed several explanations for their failure to obtain conditioning from stimulation of the central vagosympathetic trunk. Firstly, they proposed that the UCS elicited effects too extensive to form a specific CR via the autonomic nervous system. Secondly, they suggested that the UCS activated reflex pathways that were completed in the brainstem and therefore the UCS did not stimulate "higher" brain areas that theoretically provide the necessary "coupling" with the CS. Thirdly, they suggested that vagal stimulation, in contrast to a somatosensory UCS, lacked nociceptive or cognitive properties.

A large number of anatomical and electrophysiological studies support Andrus et al.'s suggestion that vagal stimulation produces extensive influences throughout a major portion of the CNS. Anatomical studies have demonstrated that the vagus nerve is composed of both motor and sensory fibers. Foley and DuBois (1937), utilizing actual fiber count, demonstrated the vagus nerve of the cat contained only 20-35% motor fibers, while the remaining were sensory. Subsequently, Agostoni, Chinnock, Daly, and Murray (1956) confirmed these findings. These investigators found that the cervical vagus of the cat contained 20-35% efferent fibers, while the remaining fibers were afferent. Similarly, Evans and Murray (1954) concluded that the rabbit vagus was composed of 20-25% efferent fibers. Comroe (1965) indicated that at least 11 types of sensory endings are served by the afferent fibers of the vagus nerve and no fewer than 12 respiratory reflexes are mediated via this nerve.

Electrophysiological studies have added support to the anatomical findings by demonstrating that vagal projections are well represented in subcortical regions. Urabe and Tsubokawa (1960) stimulated the cervical vagus of the cat and observed responses in four medullary areas: the vicinity of the solitary tract; the nucleus of the spinal tract of V; the triangular nucleus of the vestibular nerve; and the nucleus ambiguous (antidromically). Porter (1963), using single unit recording, largely confirmed the above findings. Orthodromic spikes were recorded from the tractus solitarius, nucleus of the tractus solitarius, and dorsal motor nucleus, while antidromic spikes were found in the vicinity of the nucleus ambiguous.

Considerable evidence exists to counter the second suggestion by Andrus et al. that vagal stimulation does not activate higher brain centers. Chernigouskii (1965) reviewed a number of papers that recorded evoked potentials in thalamic and cortical structures. Following direct vagal stimulation, Dell and Olsen (1951) observed evoked potentials in the region of the ventromedial, submedial, and interventral nuclei of the thalamus. Durian (1964) found representation of the vagus in two nuclei of the cat thalamus, the centrum median and dorsomedialis. These projections were overlapped by projections from somatic afferent structures. Chernigouskii and Zaraiskaya (1962) stimulated the cervical vagus nerve of the cat and observed four zones of activity, two in the orbital gyrus and two in the region of the cingulate gyrus. Recently, O'Brien, Pimpaneau, and Albe-Fessard (1970) described vagal projections in the monkey anteroventral to the tip of the rolandic fissure, a location homologous to Broca's area in humans. This area showed convergence

and interaction of short latency responses to stimulation of the vagus, larnygeal nerves, face, hands, and tongue.

The third suggestion by Andrus et al. that vagal stimulation may lack nociceptive properties is supported by a number of studies. Evans and Murray (1954) reviewed the evidence for pain fibers in the vagus and found negative results. Similarly, Pick (1970) indicated that there was no conclusive evidence of pain-conducting fibers originating in peripheral ganglia of the glossopharyngeal and vagus nerves. Cannon (1933) was one of the first investigators to chronically stimulate the vagus nerve using cats as subjects. When he stimulated in the region of the neck, coughing was so vigorous that no pertinent behavioral observations could be made. Stimulation below the recurrent laryngeal nerve reduced HR by 60-100 beats-per-minute (bpm) without disturbing the animal. Several recent studies, using vagal stimulation in chronic animals, reported no aversive effects. However, low intensity stimulation was used and behavioral observations were not essential features of these investigations (Bourde, Robinson, Suda, & White, 1970; Peñaloza-Rojas, Barrera-Mera, & Kubli-Garfias, 1969).

The above studies clearly indicate that vagal stimulation can produce extensive effects throughout the CNS. Nevertheless, there seems little reason to suppose that a specific respiratory, blood pressure, or cardiac CR could not develop. For example, electric shock produces numerous reactions in an organism, yet specific CRs can be produced using shock as a UCS. Also, studies using distension of the stomach or intestinal loop as a CS for salivary or leg-lift conditioning, indicate

that impulses from the gut, probably mediated by the vagus, can reach levels in the CNS capable of providing the necessary sensory input for the formation of conditioned connections (Bykov, 1957).

The failure of the Andrus et al. study to obtain conditioned changes may be related to the apparent absence of motivational consequences of vagal stimulation. More specifically, it is possible that vagal stimulation lacks the nociceptive properties that have been essential qualities of the UCS in other autonomic conditioning studies. It has been shown that conditioned HR acceleration in the dog is primarily mediated by a decrease in vagal activity (Dykman & Gantt, 1959; Obrist & Webb, 1967), while the conditioned HR deceleration in the rat is largely the result of increased vagal activity (Fitzgerald, O'Brien, & Martin, in press). Thus, the vagus plays an important role in mediation of conditioned HR changes in these two species. Therefore, it is conceivable that sectioning the vagus and stimulating only the cephalad end, as in the Andrus et al. study, may have eliminated processes that would normally be present with an intact nerve. Although it is impossible at this time to specify what these processes might be or how they may contribute to conditioning, their potential significance should not be overlooked.

EXPERIMENT I

The purpose of Experiment I was to examine the feasibility of using direct stimulation of the intact vagus as the UCS in the conditioning of HR deceleration in the rat. In addition to the groups receiving vagal stimulation, other groups receiving chest-shock were included to provide an estimate of the magnitude of HR conditioning that could be expected using a traditional UCS (Fitzgerald & Martin, 1971; Fitzgerald & Teyler, 1970; Teyler, 1971).

Vagal stimulation can be viewed as analogous to cortical stimulation employed to evaluate the role of motivation in skeletal conditioning. In contrast to motor cortex stimulation, however, a vagal UCS provides a minimal possibility for instrumental contingencies to affect the development of the CR. Since vagal stimulation produces consistent cardiovascular changes that reflexively and directly affect HR, blood pressure, and respiration, a contiguity interpretation of classical conditioning would predict that these changes should be capable of being conditioned when paired with an appropriate CS.

METHODS

Subjects

The subjects were 60 female, Long Evans hooded rats ranging in weight from 190-250 gms. They were purchased from Simonson Laboratories and housed in the facilities provided by the Department of Animal Care, University of Oregon Medical School. One week prior to surgery, the rats were caged individually in the laboratory under continuous illumination with free access to food and water. The water contained 0.05 mg/ml of the antibiotic Aueromycin (chlortetracycline) to minimize the possibility of infection.

Apparatus

The subjects were restrained in a U-shaped plastic restrainer with adjustable inserts positioned to hold the rats securely. The sides of the holder were slotted to allow placement of 20-ga. hypodermic needles on either side of the rat's thoracic cavity for recording the electrocardiogram (ECG). Respiration was recorded by means of an elastic mercury strain gauge wrapped around the subject's thorax. The restrainer was located in an IAC sound isolation chamber equipped with an air supply, a 10-w. house light, and a 20-cm. audio speaker mounted 15 cm. above the subject. Two rats were conditioned concurrently, each in a separate chamber, with trials alternating between rats.

Heart rate was recorded by means of an on-line system that provided a punched paper tape tabulation of HR totals for later analysis. This system, much of which has been described in detail elsewhere

(Fitzgerald, Vardaris, & Teyler, 1968), operated in the following manner. The ECG was written out on one channel of a Grass polygraph. A lever-type microswitch was positioned such that a switch closure was effected when the ECG pen was deflected by the R wave of the QRS complex. The microswitch closure triggered a pulse shaper that supplied pulses to a BCD counter. An end of count command transferred the contents of the counter to storage thus freeing the counter to accept incoming signals within 30 μsec. The stored HR total was then punched out on paper tape and the storage mode reset for the next counting period total. To provide a visual check on the reliability and accuracy of the counting circuit, the output of the pulse shaper activated another oscillograph pen. These spikes provided a record of the HR that was actually counted for comparison with the ECG record. The accuracy of the HR counting circuit was periodically checked by substituting a 10-Hz signal for the incoming ECG.

The CS was a 8-sec., 1-KHz tone, generated by an audio-oscillator, and presented through the speaker in the conditioning chamber at 75-dB sound pressure level (re 0.0002 dyne/cm^2). The CS-UCS interval was 6 sec. with the final 2 sec. of the CS being overlapped by the UCS.

The vagal UCS was a 2-sec. train of 1-msec. biphasic pulses with a frequency of 10 Hz produced by a Grass S-4 stimulator and applied to the right vagus nerve. The stimulus intensity ranged from 3.0 to 10.0 ma. and was adjusted for each subject to elicit a maximum HR deceleration without any apparent discomfort or movement. An oscilloscope equipped with a differential amplifier was used to monitor the stimulus intensity

by measuring the voltage drop across a 20-ohm resistor in series with the subject.

The chest-shock UCS was a 2-sec., 1.8-ma., 60-Hz ac shock produced by a constant-current stimulator and delivered through the ECG electrodes. A relay was used to switch the rat's ECG leads from the input of the ECG preamplifier to the output of the stimulator at the time shock occurred. Chest shock intensity was monitored using an oscilloscope equipped with a differential amplifier to monitor the voltage drop across a 100-ohm resistor in series with the rat.

Trials were initiated automatically by a film tape programmer, whereas the events within a trial were programmed and timed using Massey Dickinson transistorized logic modules.

Nerve Electrodes and Implantation

The electrodes for stimulating the vagus were constructed from two 11.0-cm. lengths of 0.25-mm. teflon coated silver wire. A 5.0-cm. silastic tube (0.18 mm. OD) was placed over the wires and knotted approximately 3.8 cm. from the end. This served to secure the wire and tubing together for later anchoring to the animal. From one end of the silastic tubing approximately 1.0 cm. of wire protruded, onto which two male Amphenol Reliatac connector pins were soldered. The pins, along with a small portion of the silastic tube were coated with Dow Corning silicone type A adhesive to form a soft plug which protruded from the animal's head and to which a female connector was attached to stimulate the nerve. The end of the electrode placed on the nerve consisted of a 0.5 cm. portion of bared wire, formed in a hook shape.

All subjects were pretreated with atropine sulfate (0.1 mg/kg) and after 15 min. were anesthesitized with sodium pentobarbital (40 mg/kg). This dosage often produced light anesthesia and the additional administration of a small amount of Pentrane (methoxyflurane) was sufficient to obtain the proper depth, while at the same time greatly reducing the loss of subjects from respiratory failure.

The surgical procedure began by making a 1.3-cm. dorsal midline incision slightly posterior to the pinna and a 3.8 cm. incision on the ventral side of the neck, extending from the posterior portion of the jaw to the anterior sternum. At this point, the silastic end of the electrode was tunneled to emerge from the head incision with approximately 1.3-cm. of the silastic portion containing the Amphenol pins exposed. The electrode was then anchored by suturing through the silastic tubing and into the surrounding neck muscles. The head incision was closed with silk suture. Beginning at the sternum and working anteriorly, the salivary glands and adipose tissue were parted. Retracting sutures were carefully placed in the superficial muscles in order to expose a thin muscle sheath which still covered the right carotid artery and vagus. At this point, aided by the use of a 13X dissecting microscope, the vagus was carefully exposed. Taking precautions not to disrupt the nerve's blood supply, the two hooks formed at the end of the electrode wire were speared under and around the nerve. A mixture of one part Dow Corning Medical Elastomer 382 and one part Medical Fluid 360 with catalyst was now applied to cover the exposed electrode wires (Straw & Mitchell, 1966). Upon hardening, the

silastic mass was further anchored by lightly tying the previous muscle sutures together. Finally, the overlying structures were returned to their proper positions and the incision was closed with 9-mm stainless steel wound clips.

The chest-shock animals received identical surgery; however, electrode wires were not attached to the nerve and the silastic mass was omitted. All subjects were conditioned on the fourth day following surgery.

Procedure

The vagal condition consisted of two groups of 15 subjects each. The vagal experimental group received paired presentations of the CS and UCS, while the corresponsing control group received unpaired presentations of the CS and UCS with the CS following the UCS by an interval of 60, 80, or 100 sec. ($\bar{X}$ = 80 sec.). A chest-shock condition comprised of an experimental and control group of 15 subjects each was included to demonstrate conditioning using a traditional UCS.

Prior to the beginning of conditioning, all subjects received a 30-min. period of adaptation to the restraining device in the sound proof chamber. At the start of this period, all animals receiving vagal stimulation were given several UCSs to adjust the stimulus intensity to elicit a maximum HR deceleration without causing any apparent discomfort to the subject. At the end of adaptation, each subject received 20 pretest CS-alone trials with an intertrial interval (ITI) of 70, 90, or 110 sec. ($\bar{X}$ = 90 sec.), followed by 40 acquisition trials with an ITI of 270, 290, or 310 sec. ($\bar{X}$ = 290 sec.).

Immediately upon completion of the acquisition trials, each animal in the vagal groups received several additional vagal stimulations. Initial observations of the effects of these stimulations were made while the subject was still restrained. Next, the subject was released and stimulated during exploration of the conditioning chamber. These procedures allowed the experimenter to determine if the nerve stimulation elicited any vocalization, locomotion, or disrupted ongoing behavior.

The HR response to the CS on the pretest CS-alone trials and on the acquisition trials was obtained by subtracting the number of heart beats during the 6 sec. immediately preceding the onset of the CS (pre-CS period) from the number during the 6-sec. CS-UCS interval. The resulting difference scores were then converted to a bpm index. All HR transformations and analyses were performed on a Wang 700 calculating system.

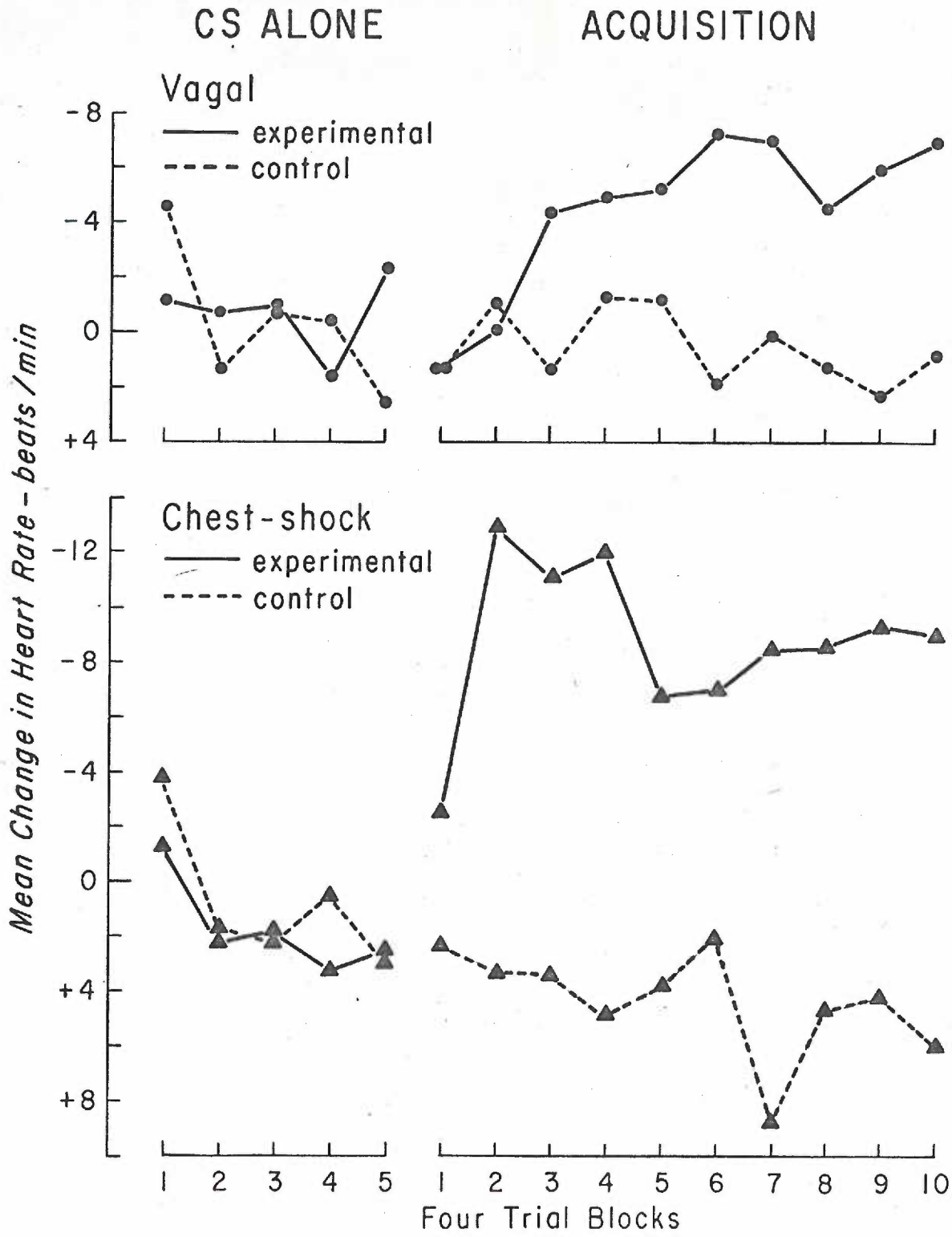
RESULTS

The pretest CS-alone and acquisition difference scores averaged over blocks of four trials are presented in Figure 1 for the vagal and chest-shock groups.

Examination of the pretest CS-alone data plotted on the left side of the figure reveals that initially all the groups showed a decrease in heart rate to the CS. It can be seen that the decelerative reaction of the vagal experimental group failed to completely habituate, while that of the vagal control group gradually changed to an increase in HR. Inspection of the results of the chest-shock experimental and control groups reveals that both groups changed from a decelerative to an accelerative response to the CS. Separate analyses of variance were performed on the difference scores of the vagal and chest-shock groups. The experimental versus control factor was not reliably different for either condition. A significant vagal experimental versus vagal control X trials interaction demonstrated that the two groups changed differently during the pretest CS-alone phase ($\underline{F} = 2.48$, $\underline{df} = 4/112$, $\underline{p} < 0.05$). Analysis of the results of the chest-shock groups revealed a reliable trials effect ($\underline{F} = 3.04$, $\underline{df} = 4/112$, $\underline{p} < 0.05$) which reflected the change in the HR response from a deceleration to an acceleration.

The upper right hand portion of Figure 1 shows the acquisition results of the vagal experimental and control groups. It can be seen from this part of the figure that vagal stimulation was an adequate UCS for the development of a conditioned HR response. For the first eight

Figure 1. Mean pretest CS-alone and acquisition difference scores of the vagal experimental, vagal control, chest-shock experimental, and chest-shock control groups averaged over blocks of four trials. The HR response to the CS was obtained by subtracting the number of heart beats during the 6 sec. immediately preceding the onset of the CS (pre-CS period) from the number during the 6-sec. CS-UCS interval. The resulting difference scores were then changed to a bpm index.


CS ALONE
ACQUISITION
Vagal
experimental
control
-8
-4
0
+4
Chest-shock
experimental
control
-12
-8
-4
0
+4
+8
Mean Change in Heart Rate - beats/min
1 2 3 4 5
1 2 3 4 5 6 7 8 9 10
Four Trial Blocks

trials, the HR responses of the vagal experimental and control groups were not different. However, after the eighth trial, the vagal experimental group developed a decelerative CR that increased in magnitude over the next 12 trials, reaching a terminal level of approximately 6 bpm. This portion of the figure also illustrates that the vagal control group developed a slightly accelerative response to the CS that showed little change during acquisition. A two-factor analysis of variance performed on the results of the vagal groups established that there was a reliable experimental versus control effect ($F = 8.89$, $df = 1/28$, $p < 0.01$), a reliable change across trials ($F = 1.96$, $df = 9/252$, $p < 0.05$), and a reliable experimental versus control X trials interaction ($F = 2.46$, $df = 9/252$, $p < 0.05$), indicating that the HR responses of the two groups changed differently across the acquisition trials.

The lower right hand section of Figure 1 presents the acquisition results of the chest-shock experimental and control groups. An examination of these data reveals that the experimental group rapidly developed a decelerative CR of approximately 12 bpm that in the second half of acquisition decreased to 8 bpm. Thus, the CRs of the chest-shock experimental group were in the same direction but slightly larger in magnitude than the CRs shown by the vagal experimental group. Further examination reveals that the chest-shock control group developed an accelerative response to the CS that gradually increased over the acquisition trials, reaching a terminal level of approximately 4 bpm. An analysis of variance on the chest-shock data demonstrated a significant experimental versus control effect ($F = 12.11$, $df = 1/28$, $p < 0.01$) and a significant

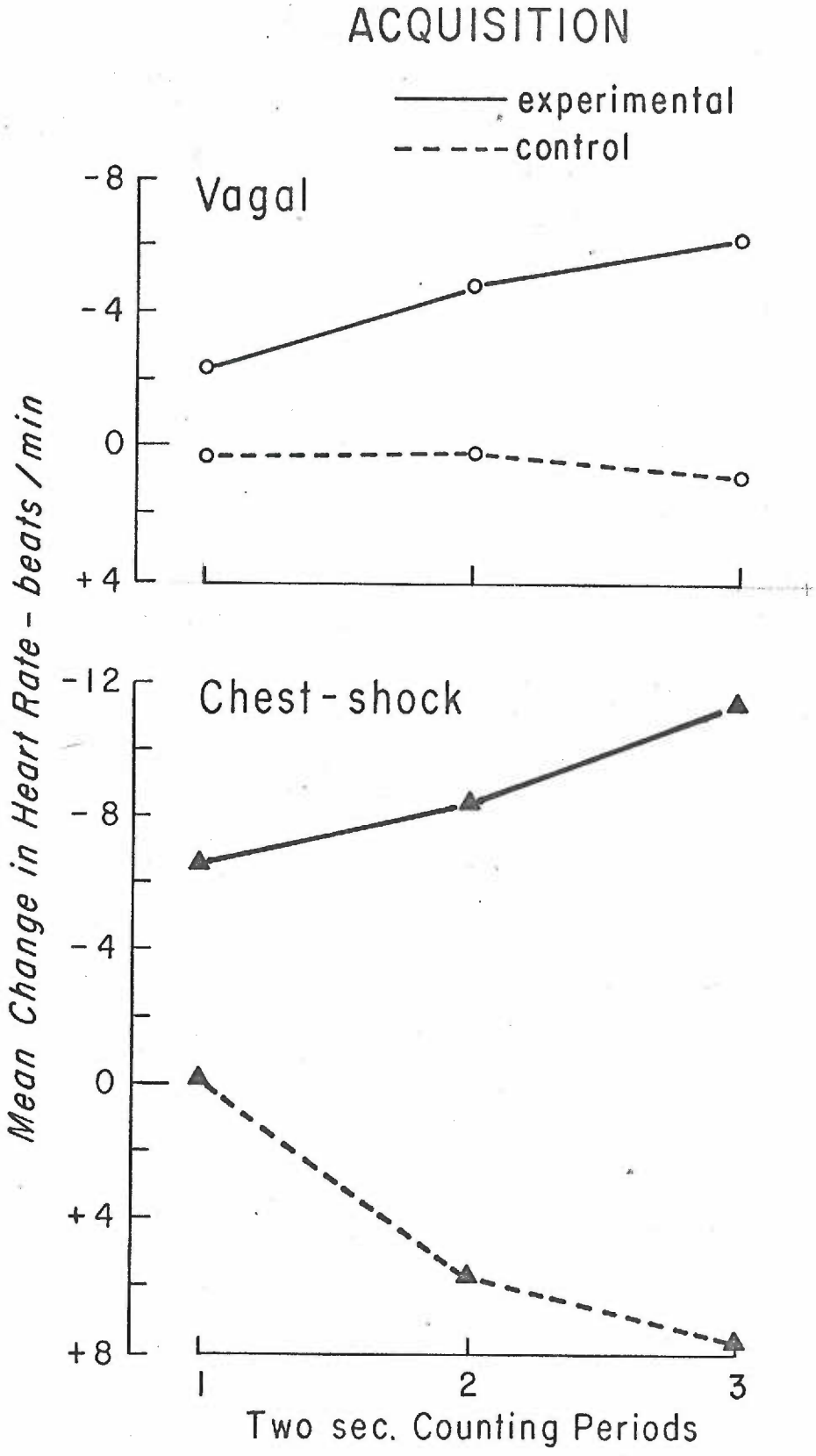
experimental versus control X trials interaction ($F = 2.02$, $df = 9/252$, $p < 0.05$), indicating that the change in the HR response across trials was different for the two groups.

It has been demonstrated, using shock as the UCS, that the decelerative HR CR typically reaches its greatest magnitude just prior to the time shock is delivered. In order to determine if a similar effect may have occurred in the present study, the form of the HR CR was analyzed by dividing the 6-sec. CS-UCS interval into three successive 2-sec. periods. A mean bpm score was tabulated for each 2-sec. period. Each of these three scores was then corrected for base level by subtracting the mean bpm HR derived from the 6-sec. pre-CS period.

Figure 2 presents the mean acquisition difference scores of all the groups for each 2-sec. period during the CS-UCS interval. The results of the vagal experimental and control groups are plotted in the upper portion of the figure. Inspection of this part of the figure reveals that the vagal experimental group showed a progressively larger deceleration toward the onset of the UCS. In contrast, it can be seen that the vagal control group showed essentially no change in response to the CS. A two-way analysis of variance on the results of the vagal groups established that there was a reliable experimental versus control effect ($F = 8.83$, $df = 1/28$, $p < 0.01$) and a reliable experimental versus control X periods interaction ($F = 5.08$, $df = 2/56$, $p < 0.01$), reflecting the difference in the form of the response of the two groups.

The response to the CS of the chest-shock groups is plotted in the lower portion of Figure 2. Inspection of this portion of the figure

Figure 2. Mean acquisition difference scores of the vagal experimental, vagal control, chest-shock experimental, and chest-shock control groups for each 2-sec. period during the CS-UCS interval. The HR response during each 2-sec. period was obtained by tabulating a mean bpm score for each period. These scores were then corrected for base level by subtracting the mean bpm HR derived from the 6-sec. pre-CS period.

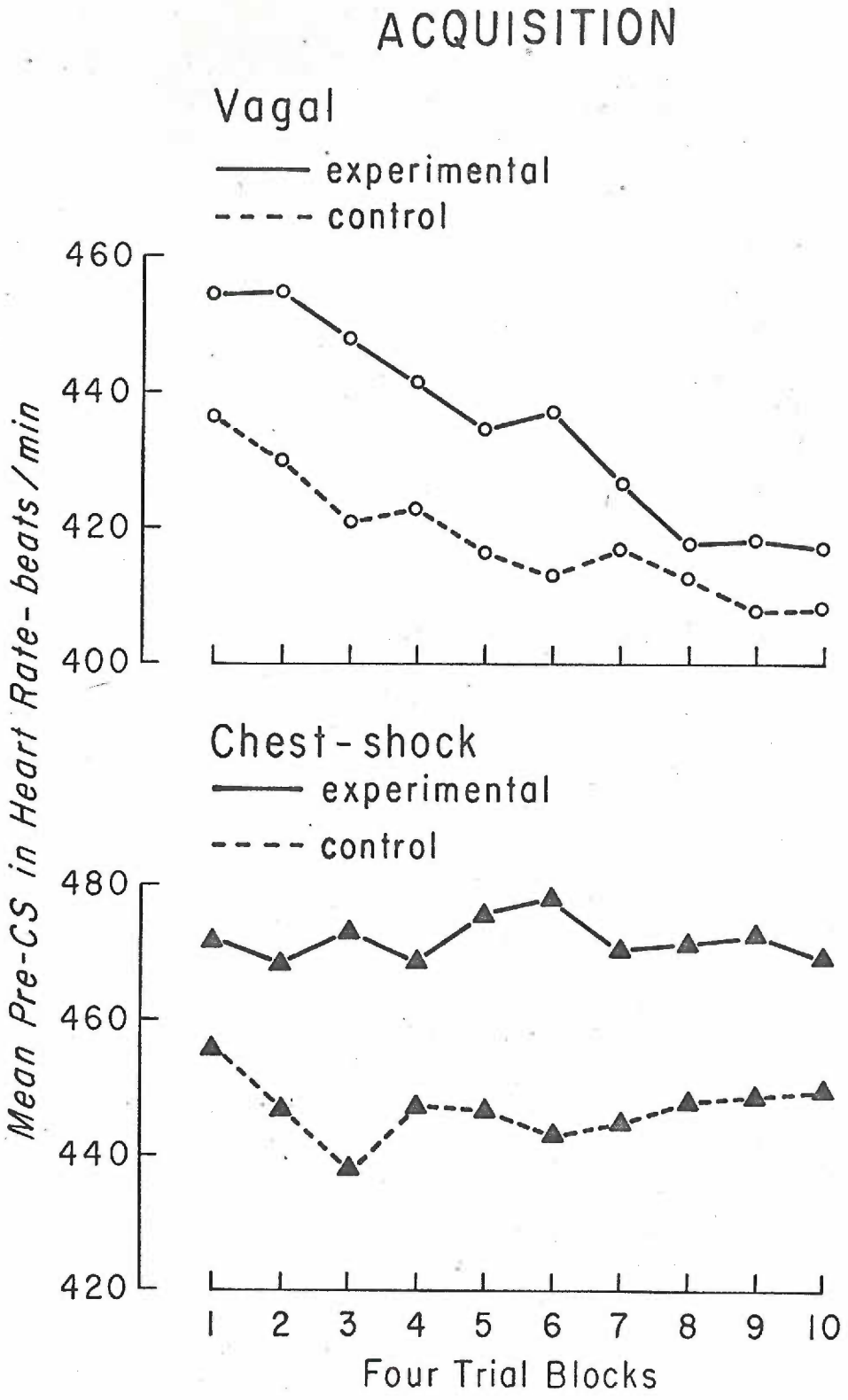

ACQUISITION
experimental
control
Vagal
-8
-4
0
+4
Chest-shock
-12
-8
-4
0
+4
+8
Mean Change in Heart Rate - beats / min
1
2
3
Two sec. Counting Periods

reveals that for the experimental group the largest deceleration occurred in the period just preceding the onset of the UCS, a result highly similar to that observed in the vagal experimental group. It can be seen that the HR response of the chest-shock control group was primarily accelerative and that this response increased in magnitude over the three counting periods. An analysis of variance on the results of the chest-shock groups revealed a significant experimental versus control effect ($F = 12.71$, $df = 1/28$, $p < 0.01$) and a significant experimental versus control X periods interaction ($F = 10.70$, $df = 1/28$, $p < 0.01$).

An overall change in base level HR could substantially influence the direction and magnitude of the conditioned HR response. For example, a lowering of pre-CS HR could significantly limit the magnitude of the decelerative HR change to the CS. A number of factors can influence a rat's normal heart rate which can range from 300-600 bpm depending on the measurement conditions. Figure 3 shows the mean pre-CS HR of the four groups as a function of four trial blocks during acquisition. Inspection of this figure reveals that the vagal groups showed a progressive decrease in pre-CS HR across trials, while the pre-CS HR of the chest-shock groups remained relatively unchanged. These visual observations were confirmed by separate analyses of variance on the results of the vagal and chest-shock conditions. A reliable trials effect for the vagal groups ($F = 19.06$, $df = 9/252$, $p < 0.01$) demonstrated that a significant decrease in pre-CS HR occurred over the 40 acquisition trials. There was no significant effect of trials in the chest-shock groups.

The HR UCRs of all the groups were measured by subtracting the bpm HR during the 6-sec. pre-CS period from the bpm HR during five successive periods which followed the CS-UCS interval. The HR of the chest-shock

Figure 3. Mean pre-CS HR of the vagal experimental, vagal control, chest-shock experimental, and chest-shock control groups averaged over four trial blocks during acquisition. The pre-CS HR in bpm was obtained from the HR totals during the 6 sec. period immediately preceding the onset of the CS.

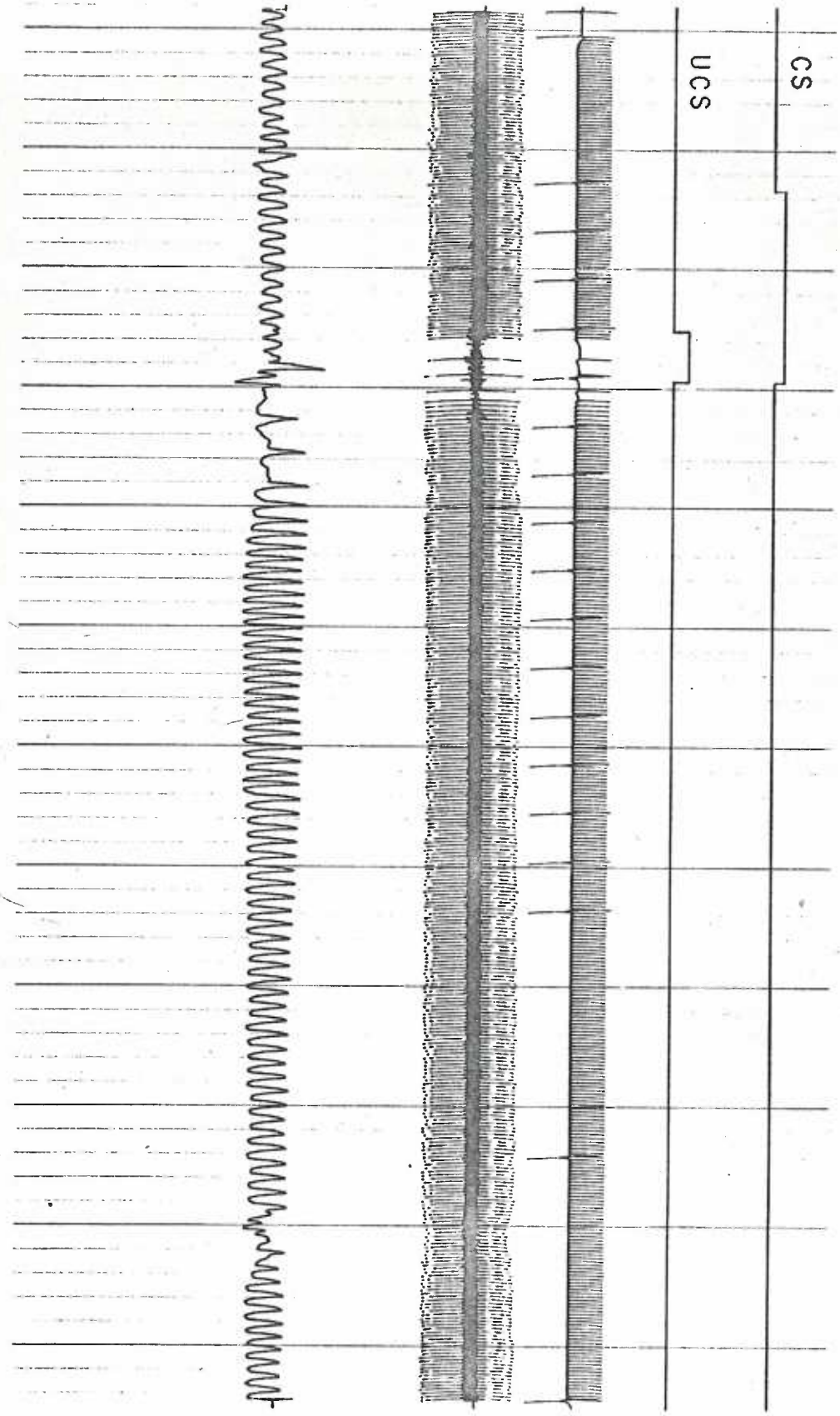

ACQUISITION
Vagal
experimental
control
460
440
420
400
Chest-shock
experimental
control
480
460
440
420
Mean Pre-CS in Heart Rate- beats/min
1 2 3 4 5 6 7 8 9 10
Four Trial Blocks

groups was switched from the input of the ECG preamplifier to the output of the shocker for delivery of the UCS. The resulting preamplifier block required that the recording of the HR UCRs of the chest-shock groups be delayed until 1 sec. after the UCS termination. For the vagal groups the HR UCRs were recorded during the presentation of the UCS.

A typical acquisition trial of a vagal experimental subject showing the UCR to vagal stimulation is presented in Figure 4. The CS and UCS signal markers are displayed on the first two channels, while the HR counting spikes and time markers are written out on the third channel. The last two channels show ECG and respiration. Inspection of the ECG and respiration channels indicates that vagal nerve stimulation produced a marked HR deceleration and hypoventilation or apnea for the duration of the stimulus. Upon the termination of stimulation, HR gradually returned toward the prestimulation base level. Respiratory frequency remained depressed for approximately 6 sec. after the offset of the UCS. Following this period of depression, respiration briefly increased in frequency and amplitude and then gradually returned toward the prestimulation level.

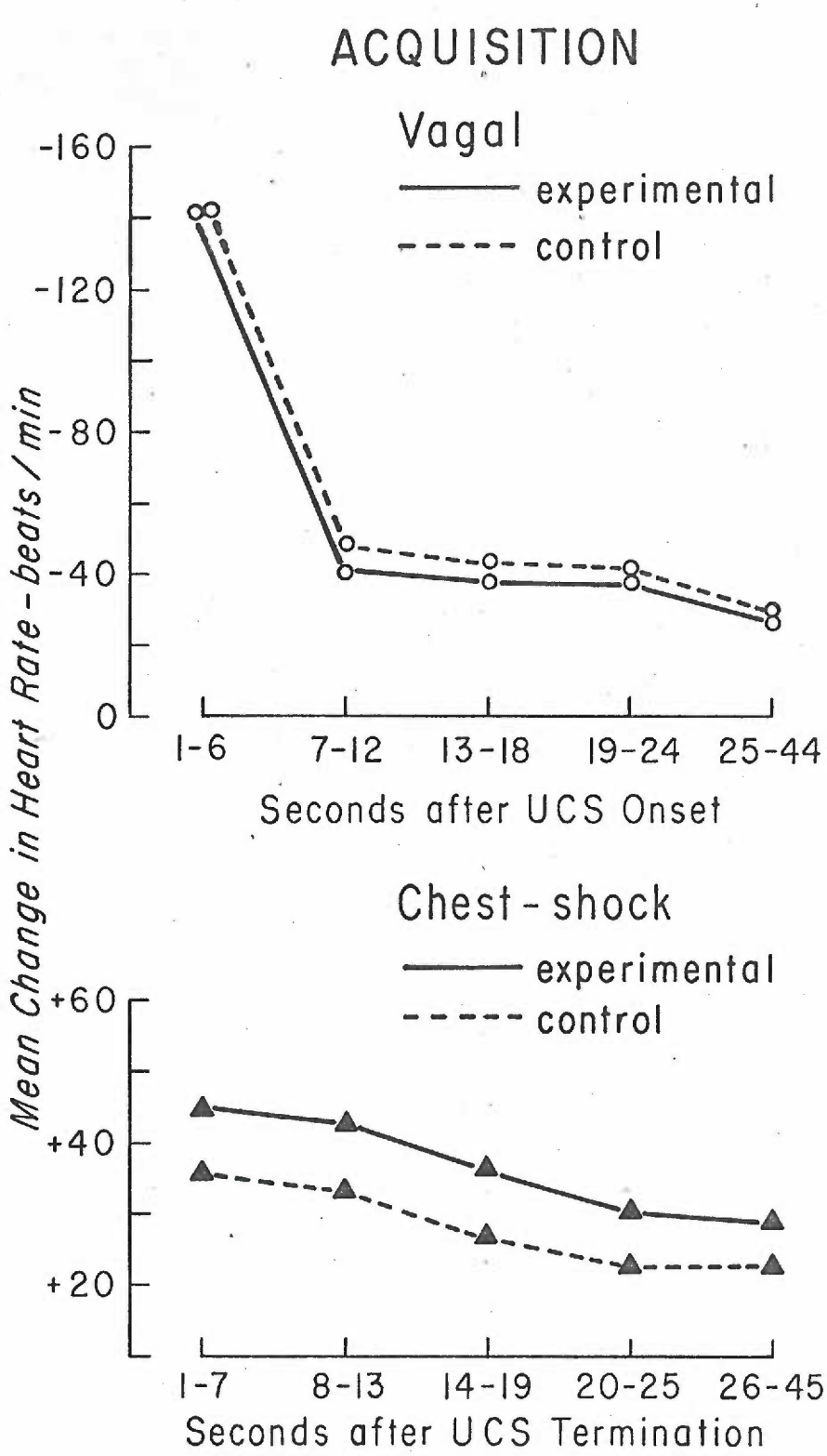
The upper portion of Figure 5 presents the mean HR UCRs of the vagal groups plotted as a function of seconds after the onset of the UCS. An examination of this part of the figure reveals that there was an initial 140 bpm decrease in HR during the first 6 sec. after the onset of the UCS. During the next 6 sec., HR increased from -140 to -40 bpm and stayed at this level for the remainder of the measurement period. This portion of the figure also illustrates that both the experimental and control groups showed almost identical UCRs. An analysis of variance

Figure 4. Sample acquisition trial of a vagal experimental subject showing the UCR to vagal stimulation. The CS and UCS signal markers occupy the upper two channels, while the HR counting spikes and time markers are written out on the third channel. The time markers set off a 6-sec. pre-CS period, three 2-sec. periods during the CS-UCS interval, 12 additional 2-sec. periods, and 2 final 10-sec. periods. The last two channels show ECG and respiration.



CONDITIONING TRIAL FOR VAGAL EXPERIMENTAL SUBJECT

Figure 5. The mean HR UCRs of the vagal experimental and control groups as a function of blocks of time after the onset of the UCS and the mean HR UCRs of the chest-shock experimental and control groups as a function of blocks of time after the termination of the UCS. The HR UCRs were measured by subtracting the bpm HR during the 6-sec. pre-CS period from the bpm HR during the five successive periods following the CS-UCS interval.


ACQUISITION
Vagal
experimental
control
-160
-120
-80
-40
0
Mean Change in Heart Rate - beats / min
1-6
7-12
13-18
19-24
25-44
Seconds after UCS Onset
Chest - shock
experimental
control
+60
+40
+20
1-7
8-13
14-19
20-25
26-45
Seconds after UCS Termination

performed on the results of the vagal groups revealed a significant periods effect (F = 154.25, df = 4/112, $p < 0.01$), indicating that the UCR changed reliably over time.

The bottom portion of Figure 5 shows the mean HR UCRs of the chest-shock groups as a function of seconds after the termination of the UCS. Inspection of this portion of the figure reveals that the UCR to chest-shock was a 40 bpm HR acceleration during the first 6 sec. after the termination of the UCS. The HR of the chest-shock groups gradually decreased over the remaining measurement periods, showing only a 25 bpm acceleration in the final period. It can also be seen from this part of the figure that the chest-shock experimental and control groups showed highly similar responses to the UCS. An analysis of variance on the results of the chest-shock groups established that there was a reliable change in the UCR over time (F = 45.33, df = 4/112, $p < 0.01$).

EXPERIMENT II

The results of Experiment I indicated that vagal nerve stimulation was a sufficient UCS for the establishment of a cardiac CR. The vagal experimental group developed a reliable decelerative CR that increased in magnitude over the acquisition trials. Analysis of the form of the response indicated that the decelerative reaction increased in magnitude toward the onset of the UCS. The CRs shown by the vagal experimental group were not as large nor did they develop as rapidly as the CRs shown by the chest-shock experimental group.

The present experiment was performed to replicate the findings of Experiment I and to determine if a second acquisition session would improve the performance level of the vagal experimental subjects. The single vagal experimental group of the present study was composed of 12 female Long-Evans rats. All the rats were treated in exactly the same manner as the vagal experimental animals of Experiment I. Following the initial 40 acquisition trials, all subjects were returned to their home cages and on the next day the rats were given 40 additional acquisition trials.

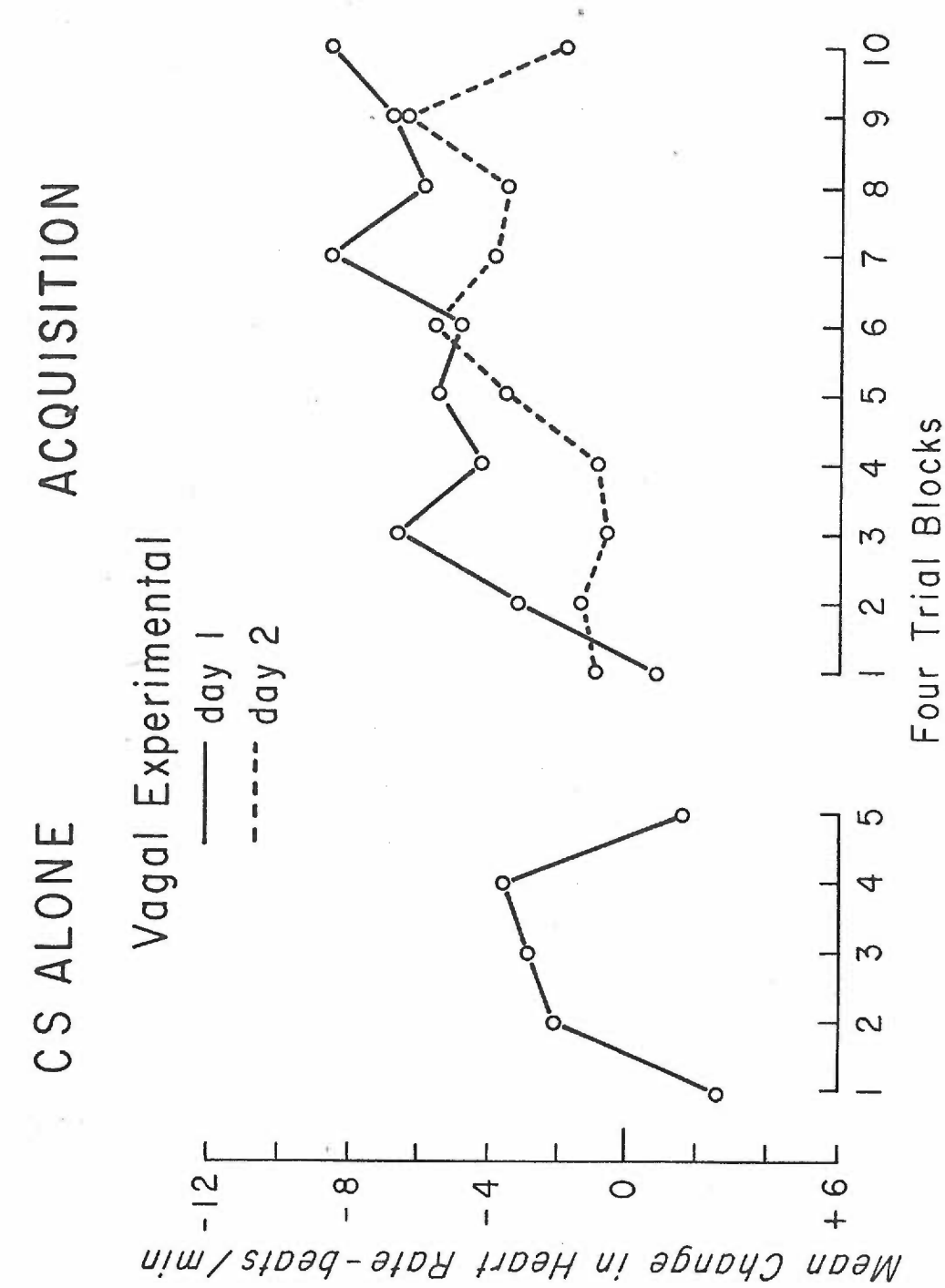
RESULTS

The HR response to the CS during the pretest CS-alone and acquisition trials was obtained by subtracting the 6-sec. pre-CS HR total from the HR total during the 6-sec. CS-UCS interval. The resulting difference scores were then changed to a bpm index. Figure 6 shows the pretest CS-alone and acquisition difference scores of the vagal experimental group as a function of four trial blocks.

The left hand portion of Figure 6 shows mean pretest CS-alone difference scores for Day 1. Inspection of this portion of the figure reveals that the initial response to the CS was a slight acceleration which became a deceleration and finally returned to an acceleration. A one-way analysis of variance demonstrated that there was a significant change across trials in response to the CS ($F = 3.03$, $df = 4/44$, $p < 0.05$).

The right hand portion of Figure 6 shows the mean acquisition difference scores of the vagal experimental group for Day 1 and Day 2. This portion of the figure shows that on Day 1 the vagal experimental subjects rapidly developed a decelerative CR that increased in magnitude during acquisition, reaching a terminal level of approximately 6 bpm. Comparison of the Day 1 curve of Figure 6 with the corresponding vagal experimental curve of Figure 1 reveals that for both groups, the CR magnitude increased as a function of trials and the terminal level of conditioning was approximately the same. To obtain an estimate of the reliability of Day 1 conditioning, the data were compared to the vagal control group of Experiment I. A two-way analysis of variance revealed

Figure 6. Mean pretest CS-alone difference scores for Day 1 and mean acquisition difference scores for Day 1 and Day 2 of the vagal experimental group averaged over four trial blocks. The HR response to the CS was obtained by subtracting the number of heart beats during the 6-sec. pre-CS period from the number during the 6-sec. CS-UCS interval. The resulting difference scores were then changed to a bpm index.


CS ALONE
ACQUISITION
Vagal Experimental
day 1
day 2
Mean Change in Heart Rate - beats / min
-12
-8
-4
0
+6
1 2 3 4 5
1 2 3 4 5 6 7 8 9 10
Four Trial Blocks

a reliable experimental versus control effect (F = 12.17, df = 1/25, $p < 0.01$), a reliable change across trials (F = 1.93, df = 9/255, $p < 0.05$), and a reliable experimental versus control X trials interaction (F = 2.45, df = 9/255, $p < 0.01$), indicating that the experimental and control groups changed differently across the acquisition trials.

Inspection of the Day 2 results plotted in Figure 6 reveals that the vagal experimental group again showed a decelerative response. However, the magnitude of the CRs appeared slightly smaller than those of Day 1. A two-factor analysis of variance comparing Day 1 and Day 2 results indicated that there were no reliable differences between the two days. However, there was a reliable change across trials (F = 3.77, df = 9/180, $p < 0.01$).

EXPERIMENT III

The results of Experiment II successfully replicated the findings of Experiment I in that reliable conditioning was again obtained using vagal stimulation as the UCS. It was also found that a second acquisition session did not improve the performance level of the vagal experimental subjects. In both Experiment I and Experiment II it was assumed that the conditioned changes resulted from vagal nerve stimulation as the UCS. However, it is possible that current leakage to surrounding tissues may have contributed to the conditioned changes observed in these experiments. Therefore, a third experiment was performed to evaluate this hypothesis.

The subjects were 16 female, Long-Evans rats. The rats were divided into two groups, a neck-stimulation experimental and neck-stimulation control group. The apparatus and procedure for the present experiment was identical to that employed for the respective vagal groups in Experiment I. All subjects received 20 pretest CS-alone trials followed by 40 acquisition trials. The neck-stimulation electrodes were placed in muscle tissue adjacent to the carotid artery, approximately 2 mm from the vagus nerve. The stimulation intensity for each subject was adjusted to 10 ma. which was the highest intensity vagal stimulation used in Experiment I.

RESULTS

The HR response to the CS during acquisition was computed by subtracting the 6-sec. pre-CS HR total from the HR total during the 6-sec. CS-UCS interval. This difference score was then converted to a bpm index.

Figure 7 presents the mean acquisition difference scores of the experimental and control groups. Examination of this figure reveals that the HR responses of the experimental and control groups were very similar. Both groups showed an overall small decelerative response that increased in magnitude during the first half of acquisition and then began to decrease. A two-factor analysis of variance comparing the experimental and control groups revealed no reliable experimental or control differences ($F = 0.39$, $df = 1/14$, $p > 0.25$), nor any reliable change across trials ($F = 1.26$, $df = 9/126$, $p > 0.25$).

The HR response to neck-stimulation was measured by subtracting the bpm HR during the 6-sec. pre-CS period from the bpm HR during five successive periods following the CS-UCS interval. Figure 8 presents the mean HR UCR of the experimental and control groups as a function of seconds after the UCS termination. Inspection of this figure reveals that the UCR to neck-stimulation consisted of an initial small HR deceleration that later became a slight acceleration. A two-way analysis of variance performed on the UCR data demonstrated that there was a reliable change in the response over time ($F = 4.95$, $df = 4/156$, $p < 0.01$).

Figure 7. Mean acquisition difference scores of the neck-stimulation experimental and control groups averaged over blocks of four trials. The HR response to the CS was obtained by subtracting the number of heart beats during the 6-sec. pre-CS period from the number during the 6-sec. CS-UCS interval. The resulting difference scores were then changed to a bpm index.

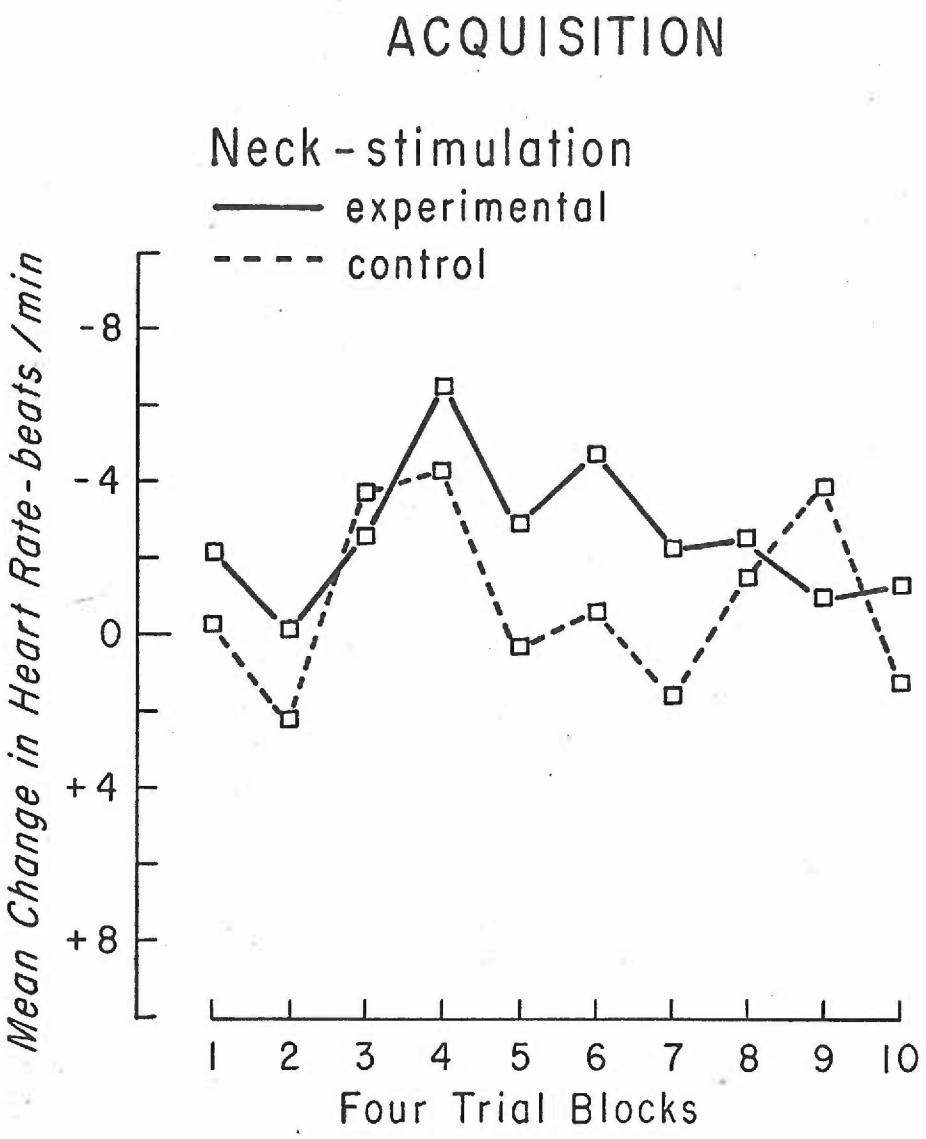
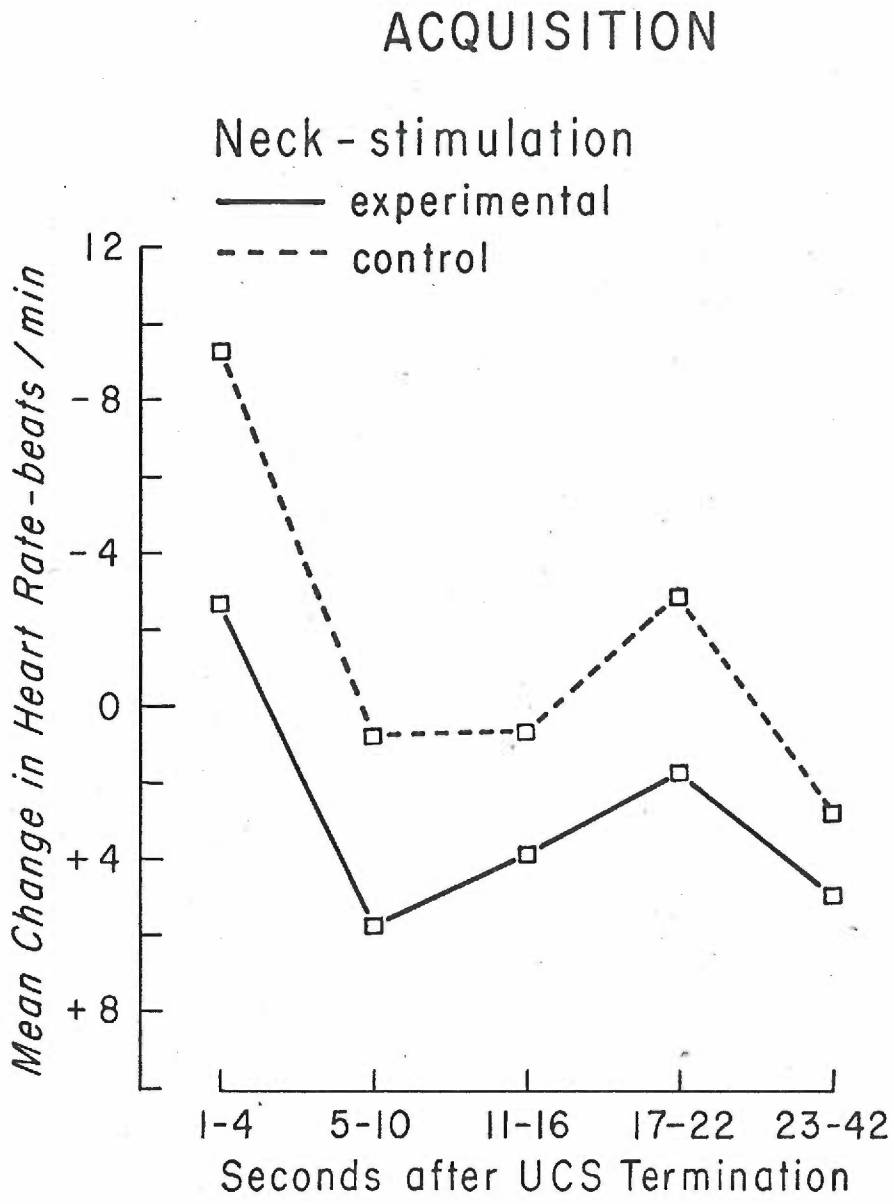

ACQUISITION
Neck-stimulation
experimental
control
Mean Change in Heart Rate-beats/min
-8
-4
0
+4
+8
1
2
3
4
5
6
7
8
9
10
Four Trial Blocks

Figure 8. Mean HR UCRs of the neck-stimulation experimental and control groups as a function of blocks of time after the termination of the UCS. The HR UCRs were obtained by subtracting the bpm HR during the 6-sec. pre-CS period from the bpm HR during the five successive periods following the CS-UCS interval.


ACQUISITION
Neck - stimulation
experimental
control
Mean Change in Heart Rate - beats / min
12
- 8
- 4
0
+ 4
+ 8
1-4
5-10
11-16
17-22
23-42
Seconds after UCS Termination

DISCUSSION

The results of Experiment I demonstrated the presence of a decelerative HR CR in rats receiving stimulation of the intact vagus nerve as the UCS. Experiment II replicated this finding and also showed that additional conditioning trials on the following day, did not increase the magnitude of the CR. The occurrence of a CR in the vagal stimulation condition is in contrast to what Andrus et al. (1969) found in the dog. Andrus et al. stimulated the cephalad end of the sectioned vagus nerve and after several hundred conditioning trials failed to find any evidence of a cardiac CR.

One possible explanation for the divergent findings is that Andrus et al. sectioned one of the vagi, whereas in the present study, both vagi were left intact. It has been demonstrated that the HR CR in the dog and the rat is primarily mediated by changes in vagal activity (Dykman & Gantt, 1959; Fitzgerald, O'Brien, & Martin, In Press; Obrist & Webb, 1967). In the case of the dog, a decrease in vagal activity is the major neural influence controlling the accelerative HR CR. It is, therefore, possible that sectioning one of the vagi interferred with the performance of the CR in the Andrus et al. study. A learned association may have been formed between the CS and cephalad stimulation of the vagus, but without both vagi, the performance of an integrated HR CR may have been prevented. In the present study, both vagi were functional and this may have substantially increased the probability of observing a vagally mediated HR CR.

A second major difference between the two studies concerns the nature of the UCR. Andrus et al. found a somewhat variable UCR consisting of both acceleration and deceleration. Typically, the vagal stimulation produced a momentary acceleration in HR that was followed by a deceleration before the stimulation ended. In several animals, the stimulation produced only HR deceleration. In the present study, stimulation of the intact vagus nerve consistently produced a substantial HR deceleration of approximately 140 bpm which may have facilitated the conditioning process.

The two studies differed in a number of other ways. Andrus et al. employed dogs as subjects, while the present study used rats. In addition, the Andrus et al. study, because of its preliminary nature, reported the methods and results in a brief manner without particular attention to procedural details. These factors make it very difficult to directly compare the results of the two studies.

The electrodes which were attached to the vagus nerve in the present investigations were not completely encapsulated by the silastic potting material. This could have allowed current to stimulate the surrounding tissues in the neck. If this stimulation was intense enough, it could have contributed to the development of the CR. Experiment III was performed to determine if direct stimulation of tissues in the neck could serve as an effective UCS for the establishment of a HR CR. The results failed to reveal the presence of a reliable HR CR. Although there was an increase in the magnitude of HR deceleration to the CS in the experimental group, it was not different from that shown by a sensitization control group. Analysis of the HR UCR to neck stimulation showed

aversive. This procedure provides one possible operational definition of aversiveness. Another technique may be to employ a shuttle apparatus where the animal can choose a stimulated or nonstimulated side (Campbell & Masterson, 1969; Sideroff et al., 1971). Behavioral observations can also provide an indication of the emotional reactions elicited by an UCS. The observation of animals receiving motor cortex or vagal stimulation indicated that these UCSs were relatively neutral. Thus, any attempt to test the motivational properties of these stimuli must be extremely sensitive. In the final analysis, failure to demonstrate any motivational properties of an UCS can be interpreted as a lack of sensitivity of the testing procedure.

In the present study, stimulation of the vagus consistently produced large changes in HR and respiration without any apparent discomfort to the subjects. After each conditioning session, the subjects were released and allowed to explore the conditioning chamber. Repeated vagal stimulation during this period did not disrupt their exploratory behavior, nor were the animals ever observed to vocalize, defecate, or show any other responses which would indicate that subjects were made fearful or anxious by the stimulation. These behavioral observations suggest that vagal stimulation produced no obvious fearful or emotional reactions. Therefore, the source of motivation typically attributed to aversive stimuli seemed not to have been present with vagal stimulation.

It has also been shown that instrumental contingencies can provide a source of motivation for the development of a classically conditioned response (Thomas, 1971; Wagner et al., 1967). Wagner et al. using motor cortex stimulation as the UCS, found that their dogs would choose

a panel that delivered a CS-UCS combination versus the UCS alone when offered the opportunity to press either panel for food reward. This finding was interpreted to mean that the CS warning was rewarding. Wagner et al. felt that the CS allowed the subject to prepare for the change in equilibrium caused by the UCR. Thus, the CR could have been reinforced by its ability to reduce the aversiveness of the change in equilibrium.

In classical HR conditioning using shock as the UCS, it is conceivable that the subject prepares for the delivery of the aversive UCS. In some manner the decelerative HR response may be reinforcing by the role it plays in these preparatory adjustments. However, it is difficult to imagine how the decelerative HR CR of the vagal experimental groups could help prepare the subject for the presentation of the UCS, or how it could instrumentally modify the decelerative HR UCR.

The present study demonstrated that vagal nerve stimulation was an adequate UCS for the establishment of a HR CR in rats. There were no indications that vagal stimulation was aversive or that it involved the elicitation of motivational processes. It can be concluded that successful conditioning using vagal stimulation as the UCS provides support for a contiguity interpretation of classical conditioning, where the essential feature is the pairing of the CS and UCS.

SUMMARY AND CONCLUSIONS

The purpose of the present studies was to determine if vagal nerve stimulation in rats could serve as an adequate unconditioned stimulus (UCS) for the establishment of a classically conditioned heart-rate (HR) response. In Experiment I the vagal experimental group received paired presentations of the conditioned stimulus (CS) and unconditioned stimulus (UCS), while the corresponding control group received unpaired presentations of the CS and UCS. A chest-shock condition comprised of an experimental and control group of 15 subjects each was included to demonstrate conditioning using a traditional UCS. The CS for both conditions was a 8-sec., 1-KHz tone, the final 2 sec. being overlapped by the UCS. The UCS for the vagal condition was a 2-sec. train of 1-msec. biphasic pulses with a frequency of 10 Hz applied to the right vagus, while the UCS for the chest-shock condition was a 2-sec., 1.8 ma., 60-Hz ac shock. All groups received 20 pretest CS-alone trials with a mean intertrial interval (ITI) of 90 sec., followed by 40 acquisition trials with a mean ITI of 290 sec.

Experiment II was performed to replicate the findings of Experiment I and to determine if a second acquisition session would improve the performance level of the vagal experimental subjects. The vagal experimental group of Experiment II received exactly the same conditioning procedures as those used in Experiment I except that a second day of acquisition training was given in Experiment II.

Experiment III was performed to determine if direct stimulation of tissues in the neck could serve as an effective UCS for the establishment

of a HR CR. An experimental and control group received the identical conditioning procedure of Experiment I, however, the UCS was stimulation of the neck tissues adjacent to the vagus nerve.

The principle findings of these studies were:

1. Vagal nerve stimulation was an adequate UCS for the establishment of a HR CR.
2. Analysis of the form of the CR demonstrated that the largest HR deceleration occurred in the period immediately preceding the onset of the UCS.
3. Behavioral observations of animals receiving vagal stimulation suggested that vagal stimulation produced no obvious fearful or emotional reactions.
4. A second day of acquisition training failed to increase the magnitude of the CR of the vagal experimental group.
5. Stimulation of tissues adjacent to the vagus nerve as a UCS did not produce a reliable HR CR.

These studies showed that classical conditioning of HR in rats can be obtained using direct vagal stimulation as the UCS even though this UCS did not elicit any obvious reactions of emotionality or fear. This finding was interpreted as providing support for a contiguity interpretation of classical conditioning.

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