EFFECTS OF INTRAUTERINE ISCHEMIA ON HEART RATE CONDITIONING IN NEONATAL DOGS

by

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INTRODUCTION

As early as the nineteenth century, W. J. Little (1861) proposed that perinatal events may be related to subsequent development and behavior. He believed that abnormal labor, premature birth, and asphyxia could affect the mental and physical condition of the child. He also suggested that asphyxia and prematurity were causes of mental deficiency. Since that time, medical, psychological, and other health professionals have devoted considerable attention to pre-, peri-, and neonatal events and their subsequent effects on the growth and development of infants. Out of this research has come the hypothesis that early hypoxic episodes may retard intellectual development. However, assessment of this possibility has been limited by a lack of fundamental knowledge about the normal learning capacities of young organisms. The general purpose of the present experiment was to provide new information on this problem area. More specifically, an attempt was made to determine the capacity of neonatal dogs to demonstrate classically conditioned changes in heart rate (HR) and to see what effects intrauterine ischemia had on HR responses.

Study of classical conditioning in young organisms has had a long history in the Soviet Union. However, only within the last two decades has classical conditioning of infants emerged as an active area of investigation in the United States. Unfortunately, many of the early Russian studies do not include details of the experimental procedures, the control conditions employed, or data analyses. The absence of this kind of information makes it difficult to compare such findings with those that have been reported more recently.

The classical conditioning paradigm was first detailed by Pavlov (1927). In general, the events which are present in classical conditioning are: (1) an unconditioned stimulus (US) which elicits a natural automatic unconditioned response (UR); and (2) a conditioned stimulus (CS) which is initially neutral in that it does not elicit the "to be conditioned" response. Following repeated pairing of the CS and US, a conditioned response (CR) begins to occur just to the CS. By adding a different CS which is never followed by a US, the classical conditioning paradigm is changed to a discrimination procedure.

The outcomes of classical conditioning studies involving human infants have been reviewed several times over the past 20 years (Brackbill, 1960, 1962; Elkonin, 1957; Kasatkin, 1972; Fitzgerald & Brackbill, 1976; Fitzgerald & Porges, 1971; Lipsitt, 1963). Experiments involving young infrahuman subjects have also been treated in several reviews (Volokhov, 1959, 1968; Volokhov, Nikitina, & Novikova, 1959). In many cases, these reviews provided data on the outcomes of Russian experiments that have not been translated into English.

The present introduction has been organized into three sections. The first two sections deal with classical conditioning studies in human and infrahuman infants, respectively. These sections are further divided into two parts, the first part dealing with skeletal motor responses and the second with reactions of the autonomic nervous system. The third section summarizes what is currently known about the behavioral effects of prenatal hypoxia.

Classical Conditioning in Human Infants

Skeletal Motor Responses

Body movements. Historically, one of the earliest indices of classical conditioning examined in human infants was that of gross changes in whole-body movements. Both Marquis (1941) and Bystroletova (1954) have reported changes in such activity using a temporal conditioning paradigm with an appetitive US. In the Marquis (1941) study, a group of infants that had been on a regular 3-hr feeding schedule for 7 consecutive days beginning on the second day of life were then shifted to a 4-hr schedule on the ninth day. In this group, a significant increase in motor activity occurred at the end of 3 hr and continued until feeding an hour later. On the ninth day, a group of infants maintained on a 4-hr schedule throughout the first 9 days showed a gradual increase in activity then a more abrupt shift in activity toward the end of the 4-hr period.

Bystroletova (1954) investigated infants during the first 9 days of their lives. The infants were divided into three groups, each receiving daily feedings on either a 3, 3.5, or 4-hr schedule. General activity, head turning, and sucking were monitored at 15 to 20-min intervals between feedings. Temporal conditioning of these reactions was detected 3 to 4 days after birth.

In a study of temporal conditioning in humans conducted by Ambrose and Lipsitt (cited in Lipsitt, 1969), HR, respiration, and movement were measured prior to the delivery of oderant, rocking, and sound USs that occurred regularly at .5-min intervals. The subjects received eight trials with each US. Test trials in which the US was omitted showed reliable conditioning of all three responses.

Wickens and Wickens (1940) tried to condition leg flexion in 3 to

5-day-old human infants. In their study, a buzzer served as the CS and a strong shock to the foot as the US. The experimental group received 12 paired presentations of the CS and US on each of 3 days. A majority of the subjects demonstrated leg-flexion CRs by the third day of training. However, a control group given US alone trials and then tested with the CS also demonstrated leg flexions. Therefore, sensitization could not be eliminated as a potential explanation of the responses shown by the experimental group.

Two investigations of leg-flexion conditioning were reported by Marum (1963, cited in Lipsitt, 1963) in his unpublished master's thesis. In the first study, 1-day-old human infants were placed in a specially constructed crib that permitted recording of general-body movements, as well as leg flexions. The CS was a tone and the US a shock administered to the toe. Twenty paired conditioning trials and five CS-alone test trials were given the experimental group on each of 4 consecutive days. A control group received the same number of CSs and USs at an extended CS-US interval. The results showed that the experimental group made significantly more responses to the CS than the control group. However, because the responses of the groups did not diverge over days, the author was reluctant to claim that learning had occurred. In the second study, 3 and 4-day-old infants were used. Each subject received CS-alone trials prior to conditioning to allow habituation of the original response to the CS. In the experimental group, these trials were followed by 50 paired conditioning trials. Reliable conditioning was not found.

Lipsitt (1963) described an experiment in which a tone CS was paired with a shock US to the toe of 3 to 4-day-old infants. Two groups were first given 10 CS-alone presentations. The experimental group then received 50 paired CS-US trials. A control group received 50 unpaired presentations of the CS and US. Positive evidence of conditioning was not obtained.

In a study in which successful conditioning of arm flexion in human infants was claimed, forced movement of the arm was used to produce the UR (Vakhrameeva, 1958, cited in Kasatkin, 1972). Infants from 2 weeks to 8 months of age received daily pairings of an air puff to the eye as the CS, followed by passive flexion of the arm. Eventually, the air-puff CS was sufficient to elicit an active arm flexion. This CR was seen in one infant who was only 19 days old. Generally, however, consistent CRs were not seen until about 4 months of age. In infants 4 months or older, CRs were more stable and were rapidly acquired.

Eyelid closures. The palpebral protective response is a reflex closing of the eyelid elicited by potentially injurious agents directed at the eye. In those studies that have attempted to condition the eyelid response, air puffs have generally been used as the US. Only two studies (Schriftsetzer, 1935, cited in Kasatkin, 1972; Wenger, 1936) were located in which visual stimulation was used as the US.

In an investigation cited in Kasatkin (1972), Nemanova (1935) found successful eyelid conditioning to a vestibular CS (i.e., rocking the infant in a cradle) that was paired with an air-puff US. Training began 10 to 13 days following birth. The CR appeared after 2 to 3 days of training. It was also reported that conditioning occurred more quickly if training began in the second month of life. In one of his own earlier studies, Kasatkin (1972) reported successful eyelid conditioning using a tone CS paired with an air-puff US. Training began around 7 days of age and continued for 2 months. Approximately 7 paired trials were given daily. Conditioned responding occurred around 30 days of age.

In contrast to these positive findings, Morgan and Morgan (1944) were unable to demonstrate eyelid conditioning in infants who were less than 54 days old. At the start of the study, the subjects ranged in age from 5 to 125 days. Each subject received up to 100 paired CS-US trials on a single day. The CS was movement of the rubber bulb that was used to present the air-puff US to the eye. Training continued until either lid closures were seen on 10 successive trials or a total of 100 trials had been presented. Infants younger than 54 days failed to demonstrate conditioning, while infants older than 66 days generally showed CRs. The authors felt that because of the number of trials given each infant, maturation was the critical factor in finding conditioning.

In a more recent study, Rendle-Short (1961), failed to find eyelid CRs in infants less than 6 months of age. Sight of the apparatus for administering the air-puff US was the CS. Each subject was only given 20 paired trials which may account for the lack of conditioning.

Lintz, Fitzgerald, and Brackbill (1967) reported eyelid conditioning in infants 33 to 133 days old (median age was 69.5 days). A tone serving as the CS was paired with an air-puff US. Test trials with the CS alone were intermixed with the conditioning trials. Twenty-five trials were given daily until CRs were present on 9 of 10 successive trials. A control group received unpaired presentations of the CS and US. Each of the experimental subjects met the criterion of conditioning, while none of the control subjects did. No information was given on the relationship between age and speed of conditioning.

Temporal conditioning of an eyelid CR was attempted unsuccessfully by Brackbill, Lintz, and Fitzgerald (1968). Each infant (mean age = 62 days) served as his own control. Initially, an air-puff US was presented at random intervals. Subsequently, each infant received the US at 20-sec intervals. Test trials on which the US was omitted provided no evidence of conditioning. In a different group of infants approximately 2 months old, eyelid CRs were obtained using an auditory CS paired with the air-puff US.

Naito and Lipsitt (1969) reported evidence of simple and differential eyelid conditioning in human infants ranging in age from 31 to 55 days of age. The experimental group received 40 paired trials of a tone CS and an air-puff US. A control group received backward conditioning trials. Intermixed among the paired trials were CS-alone test trials. Older infants tended to show a higher percentage of CRs than younger infants. In a second study, subjects 29 to 34 days of age were given differential conditioning followed by reversal conditioning. The CSs were tones of two different frequencies. The CS+ was paired with an air-puff US and the CS- was always presented alone. The mean percentage of CRs during both conditioning and reversal was higher to CS+ than to CS-, but the differences were small and not statistically reliable. The combined difference between the responses to CS+ and CS- during both phases of conditioning was reliable, suggesting that differential conditioning occurred.

A recent demonstration of eyelid conditioning in infants (median age = 36.5 days) utilized a tactile CS paired with an air-puff US (Fitzgerald & Brackbill, 1971). Movement of a thread across the baby's cheek served as the CS. During conditioning sessions, several CS-alone test trials were given. A total of 128 paired trials were given over four sessions. It appeared that conditioning was present at the end of the first session.

Two investigators used a bright light as the US to study eyelid conditioning in infant humans. The first experiment was conducted by Schriftsetzer in 1935 (cited in Kasatkin, 1972). It involved 10 to 60-day-old infants that received an auditory CS paired with the light US. Conditioning was not apparent until around 1 month of age. By the end of the second month, CRs occurred on nearly every trial.

Wenger (1936) also reported eyelid conditioning in infants who received paired presentations of a tactile CS and a bright-light US beginning the second day after birth. By the fifth day, or after about 124 CS-US pairings, conditioning occurred in three of five experimental subjects. Subjects serving as controls for maturation and sensitization were included for comparison purposes.

Eyelid conditioning has also been found in infants born prematurely (Irzhanskaia & Felberbaum, 1967; Kasatkin, 1936, cited in Kasatkin, 1972). In the Kasatkin study, infants born 1 to 2 months prematurely received paired presentations of an auditory CS and an air-puff US beginning 3 to 6 weeks following birth. The CR was first observed at about 6 weeks of age. The design of this experiment did not allow assessment of whether the number of conditioning trials, maturation, or both were factors in the emergence of the eyelid CR.

In the Irzhanskaia and Felberbaum (1967) study, infants premature by 1 to 2.5 months began training at 1.5 to 2.5 months of age. The CS was either a mint or anis odor paired with a weak air-puff US. Successful conditioning occurred to both CSs. Additional tests showed that when a nipple was saturated with the mint odor and presented during normal nursing for 2 weeks prior to conditioning, the rate of acquisition of the CR to the mint odor increased. The rate of acquisition of the CR and the percentage of CRs elicted were dependent upon the age at which training began and the degree of immaturity at birth. The older infants and those most mature at birth showed the best performance.

Mouth movements. Sucking responses have frequently been used as a measure of learning in appetitive conditioning studies of the human infant. However, it is important to point out that these responses are easily elicited in infants by a wide variety of stimuli and proper controls are needed to rule out nonassociative explanations of experimental outcomes.

Conditioned mouth-opening movements and sucking have been observed in infants prior to feeding when the feeding schedule was unchanging and predictable (Bekhterev & Shchelovanov, 1925; Denisova & Figurin, 1929; Koltsova, 1958; each of these studies cited in Kasatkin, 1972). In these experiments, the CS consisted of swaddling and then placing the infant in a natural feeding position. Bottle feeding served as the US. In these investigations, anticipatory sucking responses were found around 15 days of age (Bekhterev & Shchelovanov, 1925), around 24 days of age (Denisova & Figurin, 1929), and around 14 days of age (Koltsova, 1958). The reasons for the discrepancies between the studies probably reflect procedural differences.

Marquis (1931) gave infants repeated trials of a buzzer CS paired with the presentation of a bottle of milk. This procedure was carried out for 10 days following birth. Control infants received unpaired presentations of the buzzer and bottle. A majority of experimental infants showed sucking and mouth opening responses to the CS within 5 days, while none of the control infants demonstrated these responses to the CS.

Two studies by Kasatkin and Levikova (1935a, 1935b), showed appetitive conditioning of sucking in the human infant. In the first study (Kasatkin & Levikova, 1935a), the subjects received 6 to 12 daily pairings of a tone CS and a milk US. The conditioning began when the subjects were 11, 25, or 31 days of age. Conditioned sucking was first noticed between 35 and 44 days of age. Its occurrence did not seem to depend upon the age at which training began or the total number of trials that had been given to that time. Therefore, maturation was thought to be the critical factor in the acqusition of the CR. In the second study by Kasatkin and Levikova (1935b), simple appetitive conditioning and more complex discrimination conditioning were investigated in a group of infants who began training between the second and seventh week of life. Colored lights served as the CSs and milk presentations as the US. Regardless of when training began, each of the infants acquired a simple conditioned sucking response to a single colored light (CS+) around 58 to 62 days of age. Following the development of this simple CR, a different colored light (CS-) was tested without being followed by milk. Initially, sucking occurred to both lights, but by the third to fourth month of life, CRs occurred only to the CS+.

A vestibular CS, crib rocking, was paired with milk ingestion in a study by Nemanova (1935) that was cited by Kasatkin (1972). Conditioning trials were begun at 10 to 13 days of age, and the first sucking CRs appeared about 1.5 weeks later. Conditioned responses were more consistent in the second month of life than earlier.

Kantrow (1937) devised an ingenious method for pneumatically recording sucking responses with a chin harness whose motion was recorded on a polygraph. Human infants, 1.5 to 4 months old, were given paired presentations of a buzzer CS and a bottle US. After 16 to 72 trials, anticipatory sucking responses were consistently elicited during the buzzer alone phase of each trial. Repeated presentations of the buzzer without the US resulted in extinction of the CR.

Investigation of conditioned sucking was carried out by Lipsitt and Kaye (1964) in infants who began training at 3 to 4 days of age. In the experimental group, a non-nutritive nipple was inserted into each baby's mouth concurrently with a tone CS. Control group infants received the nipple well after the tone had been turned off. Both groups received 25 training trials, every fifth trial being a CS-alone test trial and then a number of extinction trials. The experimental group demonstrated an increase in sucking to the CS during the conditioning phase, and significant levels of responding during extinction.

Lipsitt, Kaye, and Bosack (1966) reported that conditioned sucking in 2 to 4-day-old infants occurred when dextrose solution was used as the US. The sequence of trials was as follows: 6 CS-alone trials, 10 conditioning (or comparable control trials), 10 extinction trials, 5 reconditioning trials, and 5 extinction trials. In the experimental group, on each conditioning and reconditioning trial, a 1 cc solution of

5% dextrose water was administered following the presentation of a non-nutritive nipple CS. Control infants were also given the same volume of solution but it was never paired with the non-nutritive nipple CS. The data provided evidence that sucking responses increased to the CS in the paired experimental group.

Kaye (1967) later extended the study of conditioned sucking in infants using the opportunity to suck on a non-nutritive nipple as the US. The infants in the experimental group were 3 to 4 days old, and they received five CS-alone trials, 20 training trials, and 20 extinction trials. During the training trials, a tone CS overlapped the presentation of the nipple. The principal control group was given a like number of trials, except that the tone was not paired with the nipple. The outcome of this experiment clearly supported the contention that the pairing of the tone and nipple increased the power of the tone to elicit anticipatory sucking responses.

Three different CSs (tactile, auditory, and temporal) were used in separate groups by Abrahamson, Brackbill, Carpenter, and Fitzgerald (1970) to investigate conditioned sucking in infants. The median age of the subjects in the three groups was approximately 2 weeks. Prior to conditioning the infants receiving the tactile and auditory CSs were given pretest CS-alone trials followed by 32 conditioning trials on each of 5 days. One control group received the CS and US in a random order and another control group received CS-alone presentations throughout training. The results indicated that the experimental groups had significantly higher rates of responding to the CS than either control group. The attempt to condition sucking using a temporal conditioning procedure was not successful even after 160 conditioning trials.

Babinski and Babkin reflexes. Several studies have dealt with the conditionability of the Babinski and Babkin reflexes in human infants. The Babinski toe reflex can be elicited by slowly stroking the skin along the outer border of the sole of the foot from the heel forward. It is generally used as a test of the integrity of certain motor pathways in the brain and spinal cord. Malakhovskaya (1954, cited in Kasatkin, 1972) attempted to condition this reflex to a CS of an unspecified modality. He reported that during the second month of life the Babinski reflex could be conditioned, but not at an earlier age.

The Babkin reflex is apparently specific to human infants. It consists of rapid mouth opening ("gaping") produced by simultaneous pressure being applied to both palms of a supine, quiescent infant, whose arms are held in a flexed position. In the first attempt to condition the Babkin reflex, 2 to 4-day-old infants were studied (Kaye, 1965). The CS was movement of the arms into the flexed position followed by pressure to the infant's palms which was considered to be the US. Experimental subjects received 5 CS-alone trials, 35 paired CS-US trials, and then 15 CS-alone test trials. The control group received an identical number of CS-alone and test trials; however, during the acquisition phase they received 35 US-alone palm presses. It was found that the experimental group "gaped" more during the test trials than did the control group.

Connolly and Stratton (1969) reported successful replication of the Kaye (1965) study, and in addition, they also reported successful conditioning of the Babkin reflex to a tone CS in 2 to 4-day-old infants using similar conditioning parameters.

The most recent investigation of the Babkin reflex was carried out by Sostek, Sameroff, and Sostek (1972). They failed to obtain conditioning in infants 1 to 4 days of age at testing. The parameters of this study were similar to those used in the above experiments. The main difference was that an unpaired CS-US control group was included. The authors suggested that had an appropriate unpaired group been used in prior studies of this type, successful conditioning of the Babkin reflex may not have been found.

Autonomic Responses

Classical conditioning studies of autonomic responses in human infants have generally involved the galvanic skin response (GSR), pupillary contraction or dilation, and changes in heart rate (HR). The first reports of conditioned GSRs were those by Jones (1928, 1930) using infants 3 to 9 months of age. In these studies, the CSs were either tactile, auditory, or visual, and the US was a shock. For each type of CS, conditioned changes in the GSR were noted following only 4 to 14 paired CS-US trials. When the CS was then repeated without reinforcement, the CR extinguished after several trials.

More recently, Ingram and Fitzgerald (1974) investigated the conditionability of the GSR in 3-month-old infants using a discrimination procedure. The subjects first received several days of regular discrimination conditioning consisting of a tone CS+ paired with an air-puff US, followed by reversal discrimination training in which CS+ and CS- were reversed. Each infant received a minimum of 15 CS+ and 15 CS- trials on each day. The results indicated that learning occurred during the original discrimination phase and during reversal.

In a study of discrimination and extinction of the GSR, Ingram (1978) used 4-month-old infants assigned to different groups receiving CS-US intervals of 1.5, 3.5, 5.5, or 7.5 sec. The CSs were tones, and the US was an air puff. On each of 3 days, the subjects were given 15 CS+ and 15 CS- trials followed on a fourth day by extinction. The results indicated that conditioning was successful in the 5.5 and 7.5 sec CS-US interval groups, but not in groups receiving shorter interstimulus intervals.

Extensive study of pupillary reflex conditioning in human infants was carried out by a group of investigators headed by Y. Brackbill and H. E. Fitzgerald. This work was summarized by Brackbill, Fitzgerald, and Lintz (1967) in a monograph describing three main studies. The studies involved term infants, whose median age was approximately 53 days. The US was either light onset, producing pupillary constriction, or light offset, producing pupillary dilation. The CS was either time or time plus sound. The major findings revealed that both pupillary constriction and dilation could be effectively conditioned when time served as the CS, or when time was combined with sound as the CS. Attempts to condition the pupillary reflex to sound alone were ineffective.

In a later experiment, Fitzgerald and Brackbill (1971) investigated conditioning of the pupillary reflex in a group of infants who had a median age of 41.5 days. The CS was two strokes of a strand of monofilament line across the baby's cheek. The US was either light onset or offset. Each of the five conditioning sessions consisted of up to 64 paired trials and 18 CS-alone test trials. In contrast to their previous successes, conditioning did not occur with a tactile CS.

Further examination of pupillary conditioning was carried out by Brackbill and Fitzgerald in 1972. One experimental group had a median age of 44.5 days and the other a median age of 38.5 days. In both of these groups, conditioning trials consisted of presenting the US (an illumination change) at fixed intervals of first 20 sec, followed by sec. Test trials (no US) were randomly distributed among conditioning trials. The results indicated that conditioned dilations occurred at both US intervals, however, there was no evidence of conditioned constrictions.

Changes in HR have frequently been used in conditioning experiments with normal infants. Some work by Soviet investigators has involved premature infants. Conditioned cardioaccelerations to a complex CS were observed by Zykova (1959, cited in Kasatkin, 1972) in work with premature and term infants. Polikanina (1961) also observed the development of conditioned cardioaccelerations when a tone was paired with ammonia vapors. This CR was seen in infants born 1 to 1.5 months prematurely after 30 or so conditioning trials were distributed over 1 week.

In Appleton's unpublished masters thesis (1972, cited by Clifton, 1974b), infants 2.5 to 3.0 months of age were assigned to two groups, an experimental and a conditioning control group. The experimental group received 24 trials with a tone CS paired with a glucose US delivered from a bottle. The control group was given unpaired presentations of the CS and US in a semi-random order. Each group then received 3 extinction trials. An anticipatory HR CR did not occur in the experimental group. On the extinction trials, however, HR decelerations were observed in the experimental group during the time that the US was normally presented. This HR change was considered to reflect learning.

Forbes and Porges in a paper presented to the Society for Psychophysiological Research (1972, cited in Fitzgerald and Brackbill, 1976) attempted to condition HR changes in infants that were 1 to 3 days of age. A low-intensity tone was the CS, and a high-intensity tone was the US. The experimental group was given 12 paired CS-US presentations, and the control group received unpaired presentations. Although the experimental group did not show an anticipatory HR CR to the CS, it did show HR decelerations on extinction trials during the US period. Stampes and Porges, in an unpublished manuscript (1974, cited in Fitzgerald and Brackbill, 1976) essentially replicated these findings using slightly older infants (3 to 6 days of age) and a blinking light US.

Clifton (1974a) randomly assigned infants with a mean age of 60 hr to one of three groups: experimental, random control, or a backward control. The experimental group received 30 pairings of a tone CS with a glucose US that was sucked from a bottle. The random-control group was given the same number of CSs and USs except that they varied in a semi-random fashion. The backward group received the glucose prior to the CS. Heart rate responses did not develop to the CS in any of the groups. Once again, however, on extinction trials HR decelerations appeared in the experimental group during the time that the US was usually present.

A report of unsuccessful HR conditioning was made by Pomerleau-Malcuit, Malcuit, and Clifton in an unpublished manuscript (1972, cited in Clifton, 1974b). An experimental group of infants, 15.6 weeks of age, received 24 paired presentations of a tone CS and a cradle-rock US. Control subjects were given tones and USs that were randomly presented during each trial. There was no evidence of either anticipatory HR CRs or of HR changes to US omission on subsequent extinction trials.

Brackbill (1977) investigated the HR reactions of infants whose median age was 45 hr. Each subject was tested in both active and quiet sleep states. The CS was a low-intensity tone and the US a high-intensity tone. Randomly interspersed among the conditioning trials were CS-alone test trials. Heart rate activity was greater on CS-alone test trials in both sleep states than during control periods. During quiet sleep there was a significant increase in HR activity. In both sleep states, the changes in HR activity to the CS was primarily a cardioacceleration.

In an unusual study, Krachkovskaia (1967) demonstrated conditioned leukocytosis in human infants. The UR was an elevation in the leukocyte count produced by feeding (US). When term infants, 1 to 6 days of age, had peripheral blood leukocyte counts made on a regular basis prior to feeding, it was found that up to 5 days of age, the relationship between regular feedings and the leukocyte count was variable. By the eighth day, however, most infants demonstrated elevated leukocyte counts prior to feedings. In a smaller number of infants, a change in the feeding schedule resulted in a corresponding change in the prefeeding leukocyte count. This change took several days to occur.

Another novel conditioning procedure involving infants is that of conditioned polyuria. Zamkova and Chernousova (1952, cited in Kasatkin, 1972) reported polyuria CRs in infants about 6 months of age. Each infant had historically been fed yogurt from a bottle and porridge with a spoon. Following yogurt intake, polyuria occurred routinely. This response did not occur following porridge intake. When the infants began to suck porridge from the bottle and eat yogurt with a spoon, diuresis occurred to the porridge. According to the authors, control experiments confirmed that the diuresis was based on the mode of feeding rather than the type of food that was eaten.

Zykova (1959, cited in Kasatkin, 1972) investigated the ability of term and premature infants to acquire conditioned thermoregulatory responses. Testing of the infants, which began on the first or second day of life, consisted of placing the infant in an incubator maintained at 26^{0} C for 2 hr at the same time each day. During this time, base-level measures of rectal and cutaneous temperature, pulse rate, and respiratory rate were taken. On each of the next 5 to 6 sessions, the incubator temperature was gradually raised over the 2-hr period from 26^{0} to 35^{0} C. This increase produced unconditioned elevations in body temperature, in HR, and in respiration. Later, it was found that placing the infant in the incubator (the CS) was sufficient to elicit similar increases in these measures. The CRs occurred in both term and premature infants at about 1 week of age, and they could also be extinguished with CS-alone presentations.

Summary of Classical Conditioning Studies in Human Infants

It is difficult to draw general conclusions from the above studies concerning the precise age at which classical conditioning of somatic motor and autonomic responses can be obtained in human infants. Studies in this area generally showed that 2 to 3 days following birth, human infants were able to form certain kinds of somatic motor and autonomic CRs. At the same time, however, it should be pointed out that very few attempts have been made to study children younger than 2 days old. It seems likely that the absence of this kind of work is due to the difficulties that are encountered in working with infants that are so young.

The above studies also suggest that some responses such as sucking and mouth movements that are involved in eating may be easier to condition than are other responses such as limb movements. On the other hand, food-related responses have been studied much more frequently than other kinds of behavior. One result that bears directly on the current experiment is that anticipatory changes in the HR to CS that signalled a US were not found in several different experiments involving both appetitive and aversive USs. This outcome is at odds with the many demonstrations of HR conditioning in older subjects, both human and infrahuman.

Classical Conditioning in Infrahuman Infants

Skeletal Motor Responses

As with human infants, studies of somatic motor conditioning in infrahuman infants can be classified into two broad categories: those dealing with whole-body movements and those dealing with discrete motor movements. Unlike studies involving human infants, experiments on conditioned movement activity in infrahumans have generally not utilized an appetitive US like food but instead have employed painful electric shock.

Body movements. An avian species that reportedly demonstrates conditioned motor activity when quite young is the pigeon. Tuge and Shima (1959) stated that 6 to 12-day-old pigeons exhibited an activity CR following 1 to 9 pairings of either an auditory or visual CS with a footshock US. The CRs involved movement of the head, neck, and extremities.

Fuller, Easler, and Banks (1950) recorded general movement as their measure of conditioning in puppies. The CSs were either auditory, visual, olfactory, or tactile, and the US was a shock. Testing began 1 to 5 days following birth and continued throughout the first month of life. On each day, puppies in the experimental group received a sequence of four CS-US pairings followed by a single CS-alone trial. This sequence was then repeated. Control dogs received US-alone presentations during training and then CS-alone test trials. Conditioned responses to the auditory and visual CSs were first noted around the 18th day of life and were consistently elicited several days later. The CR to the olfactory CS was first observed on the 15th day of life and was consistently observed by the 20th day. There was no evidence of a CR to the tactile CS. Dogs that began conditioning at a later age, around 20 days, acquired the CR more quickly than dogs that began conditioning at earlier ages. Therefore, a maturation factor was thought to limit the development of CRs in young dogs.

In a study of avoidance learning (leg withdrawal) in 3-week-old puppies, James and Cannon (1952) noticed that increases in whole-body movements occurred to the CS well in advance of the establishment of the avoidance response. The increases were found following an average of 29 tone CS and shock US pairings.

Classical conditioning of motor activity in infant dogs has been demonstrated by Stanley, Cornwell, Poggiani, and Trattner (1963) using a quinine solution as the US. For both the experimental and control groups, the CS was insertion of a manometer nipple into the puppy's mouth. The experimental group received a dropper filled with quinine after the CS nipple was removed, whereas the control group had the CS nipple reinserted in the mouth. Ten trials, eight paired and two CS-alone, were given on each of 7 consecutive days beginning 1 to 5 days following birth. The experimental group showed a conditioned increase in struggling to the CS.

Mason and Harlow (1958) and Harlow (1959) reported evidence of conditioning of changes in movement in infant rhesus monkeys. Subjects in the experimental and control groups began training between 3 and 15 days of age. The experimental group was given eight paired presentations of an auditory CS and a shock US on each of the 30 days of the study. One control group received presentations of the US alone and the other control group was given the CS alone. At the end of training, the experimental group demonstrated more increases in body movements on CS-alone test trials than either of the control groups.

Green (1962) extended the previous findings. In his study, acquisition, extinction, generalization, and retention of conditioned movement was examined in three groups of rhesus monkeys that were 1, 30, or 300 days old when training began. Monkeys in all groups rapidly adapted to the tone CS and all acquired movement CRs following CS and shock US pairings. The CR had two components, conditioned crouching (CC) and conditioned activity (CA). The 1-day-old group tended to show CC behavior; the 300-day-old group displayed primarily CA behavior; while the 30-day-old group showed a mixture of both types of responding. In addition, rapid decreases in the CR levels occurred in the 1-day group during extinction. This was thought to indicate inferior conditioning in the younger monkeys.

Leg flexion. Perhaps the most novel attempt to condition leg flexion was reported by Hunt (1949) using chicken embryos. Vibration of the egg shell served as the CS, and the US was shock delivered across the yolk sac. Separate groups were given 2 days of conditioning beginning at different incubation dates. The author reported that a CR could not be observed in embryos that had not been incubated at least 15 days. The results from a variety of control groups seemed to rule out nonassociative explanations of the leg-flexion responses shown in the experimental group.

Caldwell and Werboff (1962) tested the ability of 1 to 8-hr-old rat pups to form a leg-flexion CR to a vibrotactile CS that was paired with a shock US. Four experimental groups received 80 paired presentations of the CS and US separated by either .3, .6, 1.2, or 2.4 sec. Control groups received the same number of CS and USs in a semi-random fashion. Prior to conditioning all of the rats were given CS-alone trials until such time that they failed to respond to the CS on 10 consecutive trials. All groups except the .3-sec group demonstrated relibable conditioning. The level of conditioning among the groups did not differ significantly.

More recently, Gray, Yates, and McNeal (1967) also examined the development of classically conditioned leg flexions in infant rats. The CS was a vibrotactile stimulus and the US a shock. Separate groups of rats were trained on the 1st, 3rd, 6th, or 12th days of life, and within each of these age categories, subgroups received CS-US pairings at either .3, .6, or 1.2-sec intervals. Each subject was given 80 trials with every tenth trial being a CS-alone test trial. Experimental subjects received paired CS-US presentations, and the 1-day-old controls were given CS-alone trials. In general, there was little evidence of conditioning in the youngest pups, but mean anticipatory CRs increased as a function of age. Longer CS-US intervals were also more effective in producing CRs than shorter CS-US intervals.

The outcome of the same leg-flexion conditioning study in dogs has been reported twice in the literature, once under the authorship of Fuller and Christake (1959) and again under the authorship of Cornwell and Fuller (1961). In this study, a shock US was delivered during the last portion of an air-puff CS that was directed to the back of the puppy's head. Each experimental dog received 10 paired trials each day, beginning on the fourth day of life. A control group received an equal number of CSs and USs in an unpaired order. Leg-flexion CRs in the experimental group occurred on 50% of the daily trials around 2 weeks of age and on 90% of the trials around 3 weeks of age.

Conditioning of leg flexion was examined in newborn lambs and kids by Moore (1958). Each animal was placed in a harness with the shock electrodes delivering the US attached to the legs. The CS was dimming the room lights. Twenty pairings of the CS and US were given each session. The author reported that lambs and kids as young as 4 hrs of age could form conditioned leg flexions within a single session. Many of the experimental procedures and results leading to this conclusion were not given.

Solenkova and Nikitina (1960) paired a bell CS with a shock US in an effort to establish leg-flexion conditioning in infant monkeys.

Monkeys that began training 2 to 3 weeks after birth showed CRs following 3 to 7 days of training. In the older monkeys that began training 1.5 to 3.5 months after birth, only 1 to 2 days of conditioning were necessary to establish a CR.

One study of oral appetitive conditioning in infrahuman subjects was located. In this experiment, Stanley, Cornwell, Poggiani, and Trattner (1963) used a milk US to condition sucking in 1 to 5-day-old dogs. For both the experimental and control groups, the CS was insertion of a nipple into the puppy's mouth. Shortly after removal of the nipple, the experimental group received a dropper filled with milk, whereas the control group was again given the nipple. Over the course of 7 days of training, the experimental group showed the development of a sucking CR to the nipple CS.

Autonomic Responses

Classical conditioning of autonomic responses in infrahuman infants is an area of investigation that has virtually been ignored. Of the few studies that have been done, autonomic responses were often secondary to somatic motor responses. Tuge and Shima (1959) demonstrated conditioned decreases in respiration rate in 6 to 12-day-old pigeons following 1 to 9 trials of light or auditory CSs paired with a shock US. In older pigeons, the direction of the respiratory CR was an increase in rate instead of a decrease. A second report of conditioned respiratory changes involved infant monkeys (Solenkova & Nikitina, 1960). Each monkey received an auditory CS paired with a shock US. Conditioned increases in respiration occurred prior to the development of conditioned leg flexions.

Development of vascular conditioned responses was investigated by Ovakimyan (1961), in 3 to 4-month-old puppies, utilizing a differential conditioning paradigm. The CS+ and CS- were tones of different frequencies. Two USs were tried, one being a cold water thermode that produced vasoconstriction, and the other heat that caused vasodilation. Although some evidence of conditioned vasoconstriction and vasodilation was found, differentiation between the CS+ and CS- was difficult to achieve. The slow development of differentiation was interpreted as an indication of weakness in the processes of internal inhibition during the first few postnatal months of the dogs' life.

Classically conditioned HR in puppies was examined by Fuller and Christake (1959). In an initial investigation, leg flexion but not HR was successfully conditioned in 3-week-old puppies. A second investigation involved dogs ranging in age from 3 weeks to 2 months. An air puff delivered to the back of the head was the CS, and the US was a leg shock. Paired trials produced an accelerative HR CR in dogs older than 1 month. The authors believed that the accelerative CRs were not due to skeletal motor activity associated with conditioned leg flexion because leg-flexion CRs developed before the HR CRs. Moreover, they ascertained that the HR CRs generally had shorter latencies than leg-flexion CRs. In discussing the findings, they pointed out that even though dogs younger than 1 month failed to display HR CRs, they did demonstrate unconditioned HR cardioaccelerations to the US.

Summary of Classical Conditioning Studies in Infrahuman Infants

The work reviewed in the preceding section suggests that given the right circumstances, classically conditioned somatic motor and autonomic

CRs may be obtained in infrahuman infants. Unfortunately, there has been so little work in this area that it is difficult to delineate those events that may be critical in the development of classically conditioned responses. Like the human infant studies, age, maturation, type of CS and type of US all appear to play a role in the elaboration of CRs in infrahuman infants. In the single experiment involving conditioned HR in infrahuman infants, in this case dogs, the findings were mixed. In 3-week-old dogs, no conditioning was obtained. However, in older animals ranging in age from 3 weeks to 2 months, some conditioning was found in dogs that started training when they were 1 month old.

Behavioral Effects of Prenatal Hypoxia

Hypoxia is generally defined as subnormal levels of oxygen in air, tissue, or blood. It is often erroneously confused with anoxia, which is the absence of oxygen in inspired gases, arterial blood, or tissue. In the extrauterine conditions of the neonate, hypoxic states may result from the following conditions: reduced oxygen tension as occurs in _anoxic hypoxia_ or _hypoxemia_; reductions in the oxygen transport as in _anemic_ or _hemic hypoxia_; compromised blood transport which occurs in _stagnant_ or _circulatory hypoxia_; and toxic effects on tissues that occur in _histotoxic hypoxia_. The hypoxic state _in utero_ is often an inferential diagnosis and the mechanism by which it presumably occurs may be incidental to maternal factors. Theoretically, all of the extrauterine factors that induce hypoxic states may do so in the fetus, but the complex relationship among fetal, placental, and maternal physiologies often make uncertain the mechanisms by which interventions of the normal fetal metabolic state occurs.

The reviews by Meier (1971), Tune (1964), and Van Liere and Stickney (1963) dealt with studies in which the behavioral sequelae of hypoxia were examined both during and following acute or chronic exposure to hypoxia. Investigations of behavior that are made following hypoxic exposure are concerned with the long term effects of the hypoxic episode. Experiments in which behavioral measurements are made while the organism is under the influence of O_2 deprivation are concerned with the direct effects of hypoxia on responding. In the literature that exists on postnatal behavior following prenatal oxygen deficiency, two types of animal models have emerged: the mammalian and the avian. Avian models have the attraction of packaging the embryo in a shell from which development can proceed independently of maternal influences. Mammalian models are complicated by the possible interactions among fetal, placental, and maternal tissues.

The avian model has been used by Meier and Menzle (1955) in a study in which separate groups of chick embryos were deprived of oxygen by submersion in distilled water at either the 4th, 8th, 12th, 16th, or 19th day of incubation. The durations of oxygen deprivation were set at the median lethal dose (LD_{50}) at each of the incubation ages. The authors hypothesized that oxygen deprivation would have the most pronounced effects on the 8-day group because the two measures used, jumping behavior and the optokinetic reflex, were felt to be guided principally by the visual motor system which undergoes the greatest period of development around the 8th day of incubation. Their hypothesis was supported in that the most striking effects occurred in the 8-day group.

Meier (1958) later extended this finding using a larger variety of behavioral measures. Once again, the 8-day group had the most disrupted behavior profile as indexed by measures such as maze learning, locomotion, general and directed activity, and pecking. Other experimental groups also showed behavioral effects, suggesting that systems other than the visual motor were affected by oxygen deprivation.

Although details of this work are lacking, Meier (1958) reported that chicks deprived of oxygen starting on the eighth day of incubation were inferior to controls in learning a Hebb-Williams maze. Chicks deprived prior to the eighth day of incubation were superior to controls. Meier, Bunch, Nolan, and Scheidler (1960) have also reported facilitative effects of oxygen deprivation on behavior. This will be discussed in more detail shortly.

The first study of the behavioral consequences of prenatal oxygen reduction using mammalian subjects was reported by Bunch (1952). Pregnant rats were exposed to simulated altitudes of 30,000 ft for 2 hr. Subsequently, the learning ability of 100 offspring from the hypoxic mothers and 100 offspring from control mothers was assessed in a water T-maze around the 70th day following birth. Retention was tested approximately 30 days later. The hypoxic animals were inferior in both original learning and retention when compared to offspring from control mothers.

A water T-maze was also used by Scheidler in his doctoral study (1953, cited in Meier, Bunch, Nolan, & Scheidler, 1960) in conjunction with a straight water tank, and a locomotion T-maze to evaluate the behavioral consequences of prenatal oxygen reduction. The experimental subjects were offspring of 40 rats that were decompressed to 30,000 ft for 2 hr during pregnancy. Offspring of 40 control mothers of the same stock were maintained under normal laboratory conditions throughout gestation. An examination was made of the ability of the animals to: learn and retain a complex maze problem; learn a simple discrimination problem; transfer training from one learning task to another; and relearn a task following an intervening task. In general, it was found that the experimental group was inferior to the control group. In addition, it was noted that those animals whose oxygen deprivation preceded birth by approximately 10 days had the poorest learning records.

In a follow-up study by Meier, Bunch, Nolan, and Scheidler (1960), separate groups of infant rats whose mothers had been decompressed from 2 to 21 days before giving birth, were compared with a nondecompressed control group at 64 to 67 days of age using a straight water tank and a water maze. Animals who were offspring of mothers decompressed in the 5 to 8 days gestational period consistently showed the best performance, while animals who were deprived of oxygen in the 13 to 16-day period consistently showed the worst performance. The authors suggested that two "critical periods" may exist. In one period hypoxia may be facilitative, and in the other it may be deleterious.

A similar positive relationship between test behavior and fetal age of oxygen reduction has also been reported by Vierck and Meier (1963). In this study, separate groups of pregnant mice were exposed to 95% N_2 and 5% CO_2 at gestation age of 6 to 10, 11 to 15, and 16 to 20 days. The mean duration of exposure across all groups was 8.14 min and was determined for each subject on the basis of measured seizure activity. The motor activity of the offspring of the groups was contrasted with that of the offspring of untreated controls beginning at 42 days of age.

Activity was measured on 3 consecutive days for 1 hr while the animals were in a small wire mesh cage. The results indicated that the control group and the 6 to 10-day-old experimental group increased in activity over the 3 days, while the 11 to 16 and 16 to 20-day-old groups decreased. Activity decreased in all groups within each 1 hr session.

A study by Vierck, King, and Ferm (1966) examined the effects of being in a simulated altitude of 33,000 ft for 6 hr on the offspring of 2 groups of rats exposed on the 8th or 20th day of gestation. The progeny of experimental mothers were found to be hyperactive in an avoidance shuttle box when tested 90 days after birth. Emotionality was measured with a 6 component rating scale and by measures of weight loss during avoidance training. On the basis of these indices, both of the experimental groups demonstrated hypoemotionality with the 8-day group being less emotional than the 20-day group.

Martin and Becker (1971) investigated the effects of nicotine administered throughout gestation on food reinforced lever pressing of rat progeny. A control group was given prenatal oxygen reduction to control for the possibility of nicotine related hypoxia due to blanching and constriction of uterine blood vessels supplying the fetus. A 4% oxygen level was attained by slowly reducing O_2 over a period of 37 to 41 min to gravid rats placed in an airtight chamber. Pregnant mothers were maintained at this level for an additional 5 min during each of the 21 days of gestation. Male offspring were then tested on fixed ratio, variable interval discrimination, and discrimination reversal reinforcement schedules beginning at 75 to 80 days of age. The hypoxic offspring consistently displayed inferior performance.

McCullough and Blackman (1974) presented a study of the effects of prenatal hypoxia on the offspring of pregnant rats exposed to an oxygen depleted atmosphere (13% oxygen in nitrogen) at normal pressure for 2 hr daily during the last 14 days of pregnancy. Experimental and control progeny were compared using openfield behavior beginning at 55 days of age, Hebb-Williams maze solving ability beginning at 80 days of age, and latency of eye opening. The groups were different only on the last two measures. Experimental progeny made more errors in the maze and took less time to open their eyes. The author felt that the absence of differences between the groups in openfield responding lent credence to the argument that the learning deficits in this study were not confounded by changes in emotionality.

The preceding studies make clear that prenatal hypoxia can have measurable effects on behavior using a variety of different kinds of measures. In most cases, these effects were assessed using skeletal motor responding in rather complex learning situations. No studies were located in which a Pavlovian conditioning procedure was employed, nor was information found concerning the relationship between prenatal hypoxia and the function of the autonomic nervous system. The present experiment had two objectives. The first was to determine the effects of intrauterine ischemia on the learned and reflexive HR responses of neonatal dogs. A procedure developed by Hodgkinson, Hodari, and Bumpus (1967) was used to produce ischemia *in utero*. The second objective was to provide information that would increase our basic knowledge about the process of classical conditioning in young dogs.

METHODS

Subjects

The subjects were 16 five to six week old, naive Labrador dogs raised by the Department of Animal Care of the University of Oregon Health Sciences Center. Five of the animals (three males and two females) were in the experimental group and were offspring of two pure-bred Labrador mothers who had experienced uterine ischemia throughout pregnancy (see Appendix A). The remaining animals (five males and six females) comprised the control group and came from three non-operated pure-bred mothers. All of the animals were housed with their mothers from birth throughout the experiment in a .91 m x 2.44 m indoor pen and maintained on a 12 hr, light/dark cycle. In addition to nourishment gained from nursing, the diet of the animals was supplemented daily with a can of commercial cat food. Dry food and water was available ad lib. Every effort was made to provide equivalent handling and housing conditions for the experimental and control animals.

Apparatus

The experiment was conduced in a ventilated, 61 cm x 61 cm x 80 cm, sound-attenuating chamber manufactured by Industrial Acoustics Corporation. The chamber was equipped with a 60 W houselight recessed in the ceiling, two 10 cm speakers mounted on the front wall, and a 10 cm speaker mounted on the back wall. The dogs were suspended in a canvas jacket from the ceiling of the chamber approximately 12 cm back from the front speaker. To help mask extraneous auditory signals, white noise measuring approximately 80 dB sound pressure level (re. 20 $\mu N/M^2$ - measured at the approximate position of the animal's head when suspended in the sling) was continuously presented through the 10 cm speaker mounted on the back wall.

The electrocardiogram (EKG) was recorded on a Grass model 5 polygraph from silver-silver chloride electrodes that were taped in place and secured with an Ace bandage wrapped around the animal's midsection. One electrode was attached below the sternum and the other behind the shoulder blades. Heart rate was measured with an on-line system that provided paper-tape tabulations of heart-beat totals occurring in successive time intervals of each trial. This system which has been described in detail by Fitzgerald, Vardaris, and Teyler (1968), consisted of a low-force lever-type Microswitch positioned on the frame of the Grass polygraph so that the switch was activated when the EKG pen was deflected by the R wave of a QRS complex. Closure of the Microswitch triggered a transistorized pulse shaper whose output was fed into a transistorized counting network. At the end of predetermined time intervals within each trial, the cumulated heart-beat totals were punched on a Tally eight-bit high-speed paper-tape punch. The accuracy of the system was periodically checked by substituting a 10 Hz signal for the EKG signal.

The CSs were 10 sec, 700 and 1500 HZ tones produced by audio oscillators and delivered at 85 dB sound pressure level (re. 20 $\mu N/M^2$ - measured in the same way as the white noise) through the speakers located in front of the animal. The US was a 1 sec train of 100 V dc, .5 msec pulses at a frequency of 50 Hz, produced by a constant wattage shock generator and delivered through stainless steel electrodes 2 cm in diameter that were taped to the upper thighs of the hind legs. Before attaching the electrodes the thighs were shaved and electrode paste was rubbed into the skin. Resistance measurements between the electrodes were made to insure that the electrode resistance was below 10 Kohms. Shock levels were monitored periodically on an oscilloscope by measuring the voltage drop across a fixed resistor in series with one of the shock electrodes. A film-tape programmer was used to initiate the trials, while time intervals within a trial were controlled by Massey Dickinson transistorized logic modules having a repeat accuracy of .05 percent.

Procedure

The uterine blood supply of the experimental mothers was modified using a surgical procedure described by Hodgkinson, Hodari, and Bumpus (1967). A midline abdominal incision was made under general anesthesia and the uterine arteries were then wrapped with snugly-fitting, but nonocclusive, teflon tubing (I.D., 1.42 mm; O.D., 2.67 mm) cut in .5 cm lengths, and secured with 3-0 silk suture. These bands were placed bilaterally between the point of emergence of the descending vesicle artery and the first medial branch to the uterus. In addition, the small branch of the ovarian artery which anastomoses with the terminal end of the uterine artery was cut and ligated bilaterally to prevent the development of collateral circulation. The experimental mothers were bred by artificial insemination at the first estrus following surgery. After the study was completed, the experimental mothers were sacrificed and autopsied. It was found that all ligatures were intact, with the teflon bands still in place and freely moveable. There was no evidence of inflammation. No surgery was performed on the control mothers.

The experiment was carried out in four successive phases. The first phase consisted of placing the animals in the restraining sling on each of 3 days, with the recording and stimulating electrodes attached. The purpose of this phase was to allow the animals to adapt to the experimental environment and to permit baseline measures of HR to be taken. Time in the sling was gradually increased from 20 min on Day 1 to 40 min on Day 2 and then to 50 min on Day 3. Ten second samples of HR were taken every 5 min throughout each adaptation period. No stimuli were presented during this phase.

During Phase 2, all animals received 16 presentations of the 700 Hz CS and 16 presentations of the 1500 Hz CS on each of two successive days. No shocks were delivered during this phase. The first eight trials on each day consisted of the 700 Hz CS and the second eight trials the 1500 Hz CS. The same sequence was then repeated. The intertrial intervals were 70, 90, or 110 sec ($\overline{x}$ = 90 sec).

In Phase 3, the animals were given discriminative classical conditioning training on each of four successive days. This training consisted of 16 trials with the 700 Hz tone (CS+) paired with the shock US and 16 nonreinforced trials with the 1500 Hz tone (CS-) presented alone. The trials were delivered in a random order with the restriction that no more than three trials of either type could occur in a row. A delay conditioning procedure was employed with the interval between the onset of the CS+ and the onset of the US being 10 sec. The intertrial intervals were the same as those used in Phase 2.

Phase 4 consisted of reversal conditioning. During this phase, the

1500 Hz tone serving as CS- in the preceding phase was now paired with shock, and the 700 Hz tone serving as CS+ was now delivered in the absence of the US. All animals were given 16 CS+ and 16 CS- trials on each of 3 days using the same random order and stimulus parameters as those employed during original conditioning in Phase 3.

A fourth day of reversal conditioning was given the animals in the control group to determine the effects of ethanol on the HR conditioning process. Seven of the animals in this group received an injection of 1.5 g/kg (30% v/v) of ethanol administered over a period of 5 min through a 21-guage butterfly cannula placed in the cephalic vein. The four remaining animals were given a comparable volume of saline. For all animals, the conditioning trials were initiated approximately 10 min after the injection.

The number of heart beats that occurred in each of the 10-sec periods during the adaptation phase were converted to beats per min (bpm) measures. The number of heart beats occurring on each of the trials during Phases 2, 3, and 4 were tabulated in 11 consecutive time intervals. The duration of the first interval was 10 sec and the following intervals 2 sec. The 10-sec interval occurred immediately before the CSs and served as a measure of pre-CS baseline HR. The next five 2-sec intervals occurred during the CSs, whereas the final five 2-sec periods occurred after the CSs. Heart-rate responses to each type of CS were calculated by subtracting the pre-CS bpm rate from the bpm rates during each of the 2-sec intervals.

All between-group analyses of variance in the present experiment were based on the unweighted means analysis described by Winer (1971).

In this procedure the computations are performed in three steps. First, a within-subjects analysis of variance is performed in order to calculate the sums of squares necessary for the error terms. Second, an analysis of variance is carried out on the cell means of the data, and the sums of squares derived from this procedure are scaled up by the harmonic mean of the cell sizes. Finally the analysis of variance table is compiled, and *F*-ratios are calculated.

RESULTS

Preconditioning Trials with the CS-alone

The mean HR responses of the experimental and control groups averaged over the 16 preconditioning trials with each CS are shown in successive 2-sec periods of the CSs in Figure 1. The HR changes were averaged over all of the trials with a given CS after it was established that there were no significant changes in the HR over trials or over the 2 days of the preconditioning procedure in either the experimental or control groups. It is clear from Figure 1 that the original or orienting HR responses (ORs) of both groups were decelerative in direction. Although the overall magnitude of the HR OR of the experimental group to the 700 Hz CS was slightly larger than that shown by the control group, a 2 by 2 by 5 (groups by CS frequency by counting periods) analysis of variance did not provide any significant main effects or interactions that would indicate reliable differences in the HR ORs of the two groups. Individual $\underline{t}$ tests established that the overall mean HR decelerations of each group averaged across the two CSs were significantly different from zero (experimental group, $\underline{t}(4) = 2.44$, $\underline{p} < .05$; control group, $\underline{t}(10) = 3.40$, $\underline{p} < .005$).

Original Discrimination Conditioning

An evaluation of the differences between the HR responses of the experimental and control groups shown in Figure 2 was made using a 2 by 2 by 4 by 5 (groups by type of CS by days by counting periods) analysis of variance. The significant outcomes of this analysis pertaining to group

Figure 1. Mean CS minus pre-CS heart-rate responses of the experimental and control groups during the five 2-sec periods of each CS (700 Hz and 1500 Hz) on the CS-alone trials.

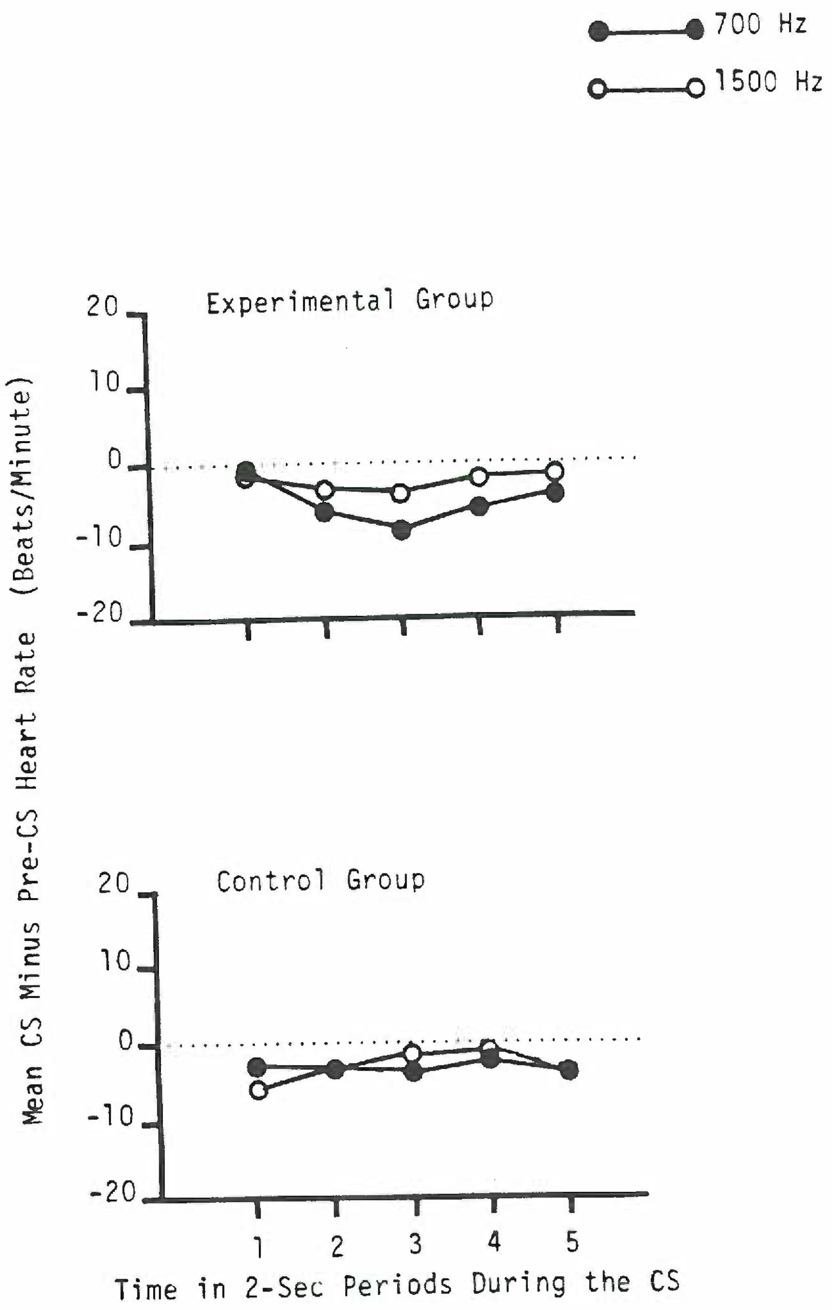
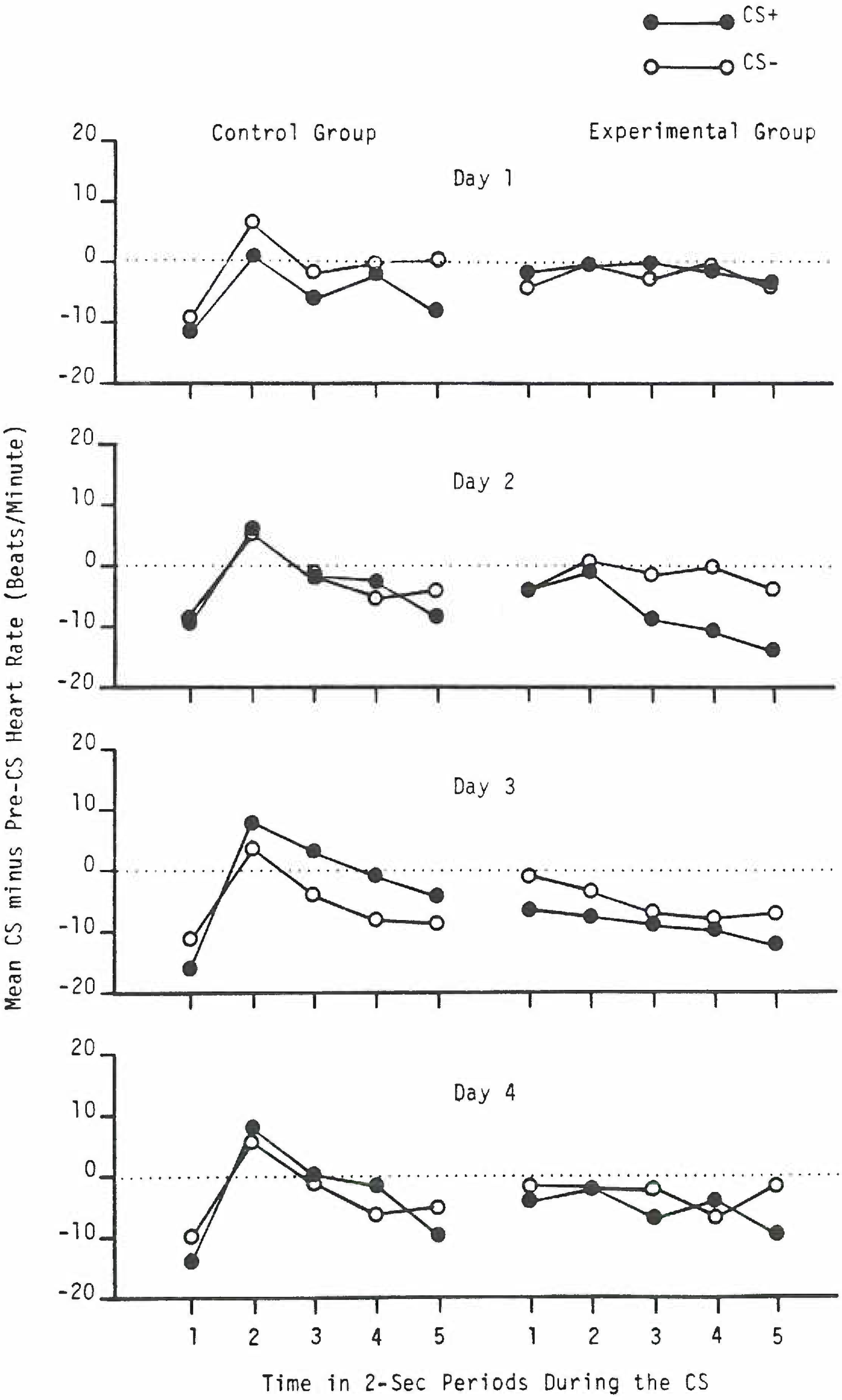

700 Hz
1500 Hz
Experimental Group
Control Group
20
10
0
-10
-20
Mean CS Minus Pre-CS Heart Rate (Beats/Minute)
1
2
3
4
5
Time in 2-Sec Periods During the CS

Figure 2. Mean CS minus pre-CS heart rate responses of both groups during five 2-sec periods of the CS+ and CS- averaged over 16 trials on each of the 4 days of original conditioning. The control group is on the left and the experimental group on the right.


CS+
CS-
Control Group
Experimental Group
Day 1
Day 2
Day 3
Day 4
20
10
0
-10
-20
Mean CS minus Pre-CS Heart Rate (Beats/Minute)
1
2
3
4
5
1
2
3
4
5
Time in 2-Sec Periods During the CS

effects were the groups by days by type of CS interaction, *F*(3, 42) = 3.78, *p* < .05, and a groups by counting periods interaction, *F*(4, 56) = 5.33, *p* < .001. Because of the high order interactions each of the groups were analyzed separately using 4 by 2 by 5 (days by type of CS by counting periods) analyses of variance.

The mean HR responses of the experimental and control groups in 2-sec periods of CS+ and CS- during each of the 4 days of original conditioning are shown on the right and left sides, respectively of Figure 2. Consistent with what was observed on the preconditioning trials, Figure 2 reveals that the directions of the HR changes of the experimental group to both CS+ and CS- were principally decelerative. There was no evidence of a consistent HR acceleration in any part of either CS. This figure also makes clear that the overall magnitudes of the experimental group's HR changes to CS+ and CS- were generally similar. Although the HR decelerations to CS+ appeared to be getting larger than those to CS- during the second day of conditioning, by the fourth day, the reactions were again equivalent. The lack of differential responding to CS+ and CS- indicating successful conditioning in the experimental group was supported by the absence of any significant outcomes in a 2 by 4 by 5 (type of CS by days by counting periods) analysis of variance.

Table 1 shows the mean HR response of each animal in the experimental group in successive 2-sec periods of the respective CSs averaged over the 4 days of conditioning. This table reveals that with the exception of Dog 3, the dominant response was HR deceleration in each period of CS+ and CS-. Also shown is the fact that in only three of the five dogs, (Dogs 1, 2, and 5) were overall mean HR decreases to CS+ larger

Table 1

Mean HR responses of animals in the experimental group during 2 second-periods of the CSs averaged over the 4 days of original conditioning.

Subject Number	Counting Periods					Mean
	1	2	3	4	5	
			CS+			
1	-8.06	-2.43	-1.50	-3.37	-9.46	-4.96
2	2.62	-9.09	-12.84	-14.71	-17.53	-10.31
3	-5.53	3.37	1.50	-1.78	-3.18	-1.12
4	-.37	2.90	-2.71	.09	-3.65	-.75
5	-7.68	-8.15	-13.31	-12.37	-11.90	-10.68
Mean	-3.81	-2.68	-5.76	-6.43*	-9.15*	-5.56*
			CS-			
1	-1.96	-2.90	-1.96	-3.84	-.09	-2.15
2	-2.15	-4.96	-9.18	-1.21	-10.12	-5.55
3	-2.81	6.09	2.34	-1.40	-3.18	+.94
4	-1.96	-2.43	-.56	-3.84	-3.65	-2.62
5	-3.75	-.46	-6.56	-7.96	-11.90	-4.95
Mean	-2.53*	-.94	-3.19	-3.66*	-4.03*	-2.87*

*$p < .05$ when compared to zero.

than those to CS-. The overall mean HR responses were quite small to both CSs in the remaining animals. Separate t tests in which the group means for each counting period were compared against zero established that the HR decreases in Counting Periods 4 and 5 were significant. The decrease in the first counting period of CS- was also significant. Finally, the overall mean HR decreases occurring to CS+ and CS- were both significantly different from zero.

Turning to the left side of Figure 2, it is clear that in contrast to the monophasic HR decelerations of the experimental group, the HR reactions of the control group to CS+ and to CS- contained both decelerative and accelerative components. With the onset of the CSs, there was first HR deceleration in Counting Period 1, followed by HR acceleration in Counting Period 2, and then a return toward HR deceleration in the remaining counting periods. As was true of the experimental group, there was little evidence of major differences between the HR responses of the control group to CS+ and to CS- during the 4 days of conditioning. The only visible evidence of differential responding was in terms of the slightly larger HR decelerations to CS+ in the first counting period and the slightly larger HR accelerations in the second counting period that appeared after the first day of conditioning. A 2 by 4 by 5 (type of CS by days by counting periods) analysis of variance provided a significant counting periods effect, $F(4, 40) = 16.59$, $p < .001$, indicating that the HR change over the CSs was reliable. There was also a significant type of CS by days interaction, $F(3, 30) = 3.79$, $p < .05$, showing that the change in the responses to CS+ and CS- was reliable. Finally, there was a type of CS by counting periods interaction, $F(4, 40) = 6.92$, $p < .001$, establishing that the HR responses over the counting periods of the CSs were reliably different.

Table 2 shows the HR reactions of each animal in the control group in successive 2-sec periods of the two CSs averaged over the 4 days of conditioning. The significant outcomes of separate t tests comparing the group means against zero are designated with an asterisk. An inspection of the table reveals that all 11 dogs displayed HR decelerations in the first counting period of both CS+ and CS-, and that these group means were significant. During the second counting period, HR acceleration occurred in nine of the dogs to CS+ and in eight of the dogs to CS-, with the group means being significantly different from zero in each case. In the remaining counting periods, the HR reactions of individual animals were somewhat variable in direction, but the overall group means were consistently decelerative.

In order to better characterize the nature of the complex HR responses that occurred to CS+ and CS- in the control group, separate trend tests were performed on the HR changes in the five counting periods of the CSs averaged over the 4 days of conditioning. These tests showed that the HR responses to both CSs contained significant quadratic components, CS+, $F(4, 40) = 46.96$, $p < .001$; CS-, $F(4, 40) = 18.54$, $p < .001$, and significant cubic components, CS+, $F(4, 40) = 15.43$, $p < .001$; CS-, $F(4, 40) = 28.92$, $p < .001$. The significant quadratic effects which accounted for 65.6% and 35.9% of the variance in the CS+ and CS- respectively, reflects the reliability of the inverted U-shaped HR response forms during the early counting periods; whereas the cubic effects, which accounted for 21.6% and 56.7% of the variance in the CS+ and CS-, respectively,

Table 2

Mean HR responses of animals in the control group during 2-second periods of the CSs averaged over the 4 days of original conditioning.

Subject Number	Counting Periods					
	1	2	3	4	5	Mean
			CS+			
1	-17.25	5.71	4.31	-.37	-6.00	-2.71
2	-8.25	8.15	-3.09	-.28	-2.62	-1.21
3	-25.87	15.37	-7.12	-8.06	-16.96	-8.53
4	-3.00	11.06	14.81	12.00	4.03	+7.78
5	-11.71	11.25	.00	-7.96	-7.96	-3.28
6	-11.15	18.84	2.90	1.96	-3.18	+1.87
7	-20.15	5.15	-1.40	-10.31	-11.25	-7.59
8	-10.78	-9.37	-10.78	-8.90	-17.34	-11.43
9	-9.28	2.90	-.84	8.53	1.50	+.56
10	-6.84	-7.78	-8.25	-7.78	-13.87	-8.90
11	-13.87	2.06	1.12	2.53	-4.96	-2.62
Mean	-12.56*	+5.76*	-.76	-1.70	-7.15*	-3.28*
			CS-			
1	-9.18	5.34	3.00	.18	-2.62	-.65
2	-3.93	5.90	-4.87	-8.62	-3.46	-3.00
3	-22.78	14.71	-3.56	-10.59	-12.93	-7.03
4	-2.81	9.84	8.43	6.56	-2.34	+3.93
5	-17.71	9.93	-6.93	-9.75	-10.68	-7.03
6	-9.75	13.68	1.03	-6.46	-1.31	-.56
7	-18.18	6.18	1.96	-5.06	-3.18	-3.65
8	-1.78	-.84	-3.65	-6.46	-1.31	-2.81
9	-3.00	-1.12	.28	-.65	.75	-.75
10	-7.03	-1.87	-7.96	-10.78	-6.56	-6.84
11	-5.25	.84	-4.78	-1.03	-2.43	-2.53
Mean	-9.22*	+5.69*	-1.55	-4.79*	-4.19*	-2.81*

*$p < .05$ when compared to zero.

indicates the reliability of later decelerative components.

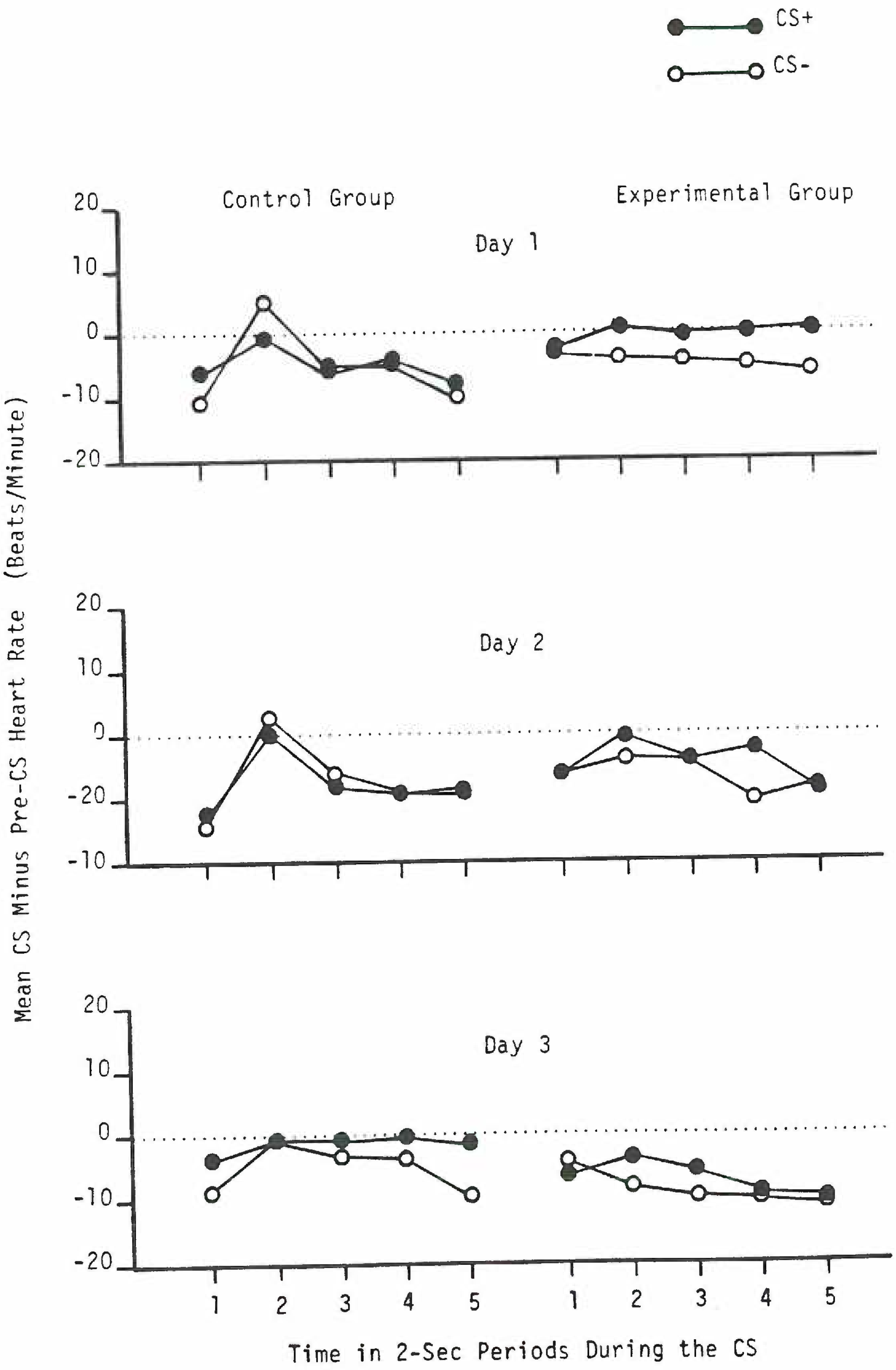
Reversal Discrimination Conditioning

A comparison of the HR responses of the experimental and control groups shown in Figure 3 indicates that the reactions of the control group became like those of the experimental group over the 3 days of reversal conditioning. In both groups, the reactions to CS- were actually slightly larger than those produced by CS+. A four-way (groups by type of CS by counting periods by days) analysis of variance provided no significant effects involving groups as a factor. Further analyses were made of each group separately.

During reversal conditioning, the CS+ that was paired with the shock US during original conditioning was presented alone without the shock, while the CS- that was given alone during original conditioning was now paired with the US. Figure 3 shows the HR reactions of the two groups to the respective CSs during each of the 3 days of reversal conditioning. From this figure, it is clear that the responses of the experimental group, shown on the right side of the figure, were similar to those displayed during original conditioning. Thus, the HR reaction to both CS+ and CS- was the dominant cardiodeceleration on each of the 3 days of conditioning. The overall magnitudes of the HR responses to both CSs increased slightly, but significantly, over days, $\underline{F}(2, 8) = 6.44$, $\underline{p} < .05$, with the responses to CS- being significantly larger than those occurring to CS+, $\underline{F}(1, 40) = 30.90$, $\underline{p} < .001$. These were the only significant outcomes from a days by type of CS by counting periods analysis of variance.

The HR responses of each of the five animals in the experimental

Figure 3. Mean CS minus pre-CS heart rate responses of both groups during five 2-sec periods of the CS+ and CS- averaged over 16 trials in each of the 3 days of the reversal training procedure. Control and experimental groups are on the left and right sides of the figure, respectively.


CS+
CS-
Control Group
Experimental Group
Day 1
Day 2
Day 3
20
10
0
-10
-20
20
10
0
-20
-10
20
10
0
-10
-20
Mean CS Minus Pre-CS Heart Rate (Beats/Minute)
1
2
3
4
5
1
2
3
4
5
Time in 2-Sec Periods During the CS

group averaged over the 3 days of reversal conditioning are presented in Table 3. As was true of original conditioning, this table makes clear that the dominant reaction of all experimental animals was HR deceleration during all periods of both CSs. Only Dogs 3 and 4 displayed instances of accelerative HR changes with these occurring in early CS periods.

Turning to the left side of Figure 3, it may be seen that the accelerative HR changes that were clearly evident during original conditioning in the control group were not retained during reversal conditioning. Although small HR accelerations occurred to CS- on the first 2 days, they were gone by Day 3, and did not occur at all to CS+. In fact, by Day 3, CS+ elicited very little HR change in any counting period. Once again, the absence of major differential responding to CS+ and CS- was confirmed by outcomes of a three-way (type of CS by days by counting periods) analysis of variance. This analysis provided a significant counting periods effect, $\underline{F}(4, 40) = 8.44$, $\underline{p} < .001$, a significant counting periods by days interaction, $F(8, 80) = 2.43$, $\underline{p} < .05$, and a significant type of CS by counting periods by days interaction, $\underline{F}(4, 40) = 6.14$, $\underline{p} < .001$. The latter interaction can be attributed to the multiple crossings of the functions for CS+ and CS- on the different days.

An overview of the main findings of reversal conditioning for the control group may be obtained by referring to Table 4 in which the mean HR responses of each control animal are shown. Clearly, the dominant reaction to both CSs was HR deceleration, with only five of the animals still showing HR accelerations in Counting Period 2. In contrast to original conditioning, neither of the mean HR changes in the second counting periods of the two CSs were significantly different from zero.

Table 3

Mean HR responses of animals in the experimental group during 2-second periods of the CSs averaged over the 3 days of reversal conditioning.

Subject Number	Counting Periods 1	2	3	4	5	Mean
			CS+			
1	-1.75	-1.75	-1.12	-.50	3.87	-.25
2	-7.50	-1.25	-5.62	-7.50	-10.62	-6.50
3	-7.12	4.12	-2.75	-2.12	-4.00	-2.37
4	-1.00	1.50	4.00	-2.87	-2.87	-.25
5	-7.62	-8.87	-15.12	-8.87	-16.37	-11.37
Mean	-5.00*	-1.25	-4.13	-4.38*	-6.00	-4.15
			CS-			
1	-2.50	-6.25	-4.37	-1.25	-2.50	-3.37
2	-5.37	-8.50	-9.12	-12.25	-16.62	-10.37
3	-8.37	1.62	-4.00	-6.50	-4.62	-4.37
4	2.25	-2.75	2.87	-4.62	-6.50	-1.75
5	-8.12	-10.62	-16.25	-17.12	-14.00	-13.22
Mean	-4.43*	-5.30*	-6.18	-8.35*	-8.85*	-6.62*

* $p < .05$ when compared to zero.

Table 4

Mean HR responses of animals in the control group during 2-second periods of the CSs averaged over the 3 days of reversal conditioning.

Subject Number	Counting Periods					Mean
	1	2	3	4	5	
			CS+			
1	-8.75	3.12	4.37	-5.00	1.25	-1.00
2	.12	-1.75	-6.12	-.50	-3.00	-2.25
3	-13.87	3.00	-12.00	-12.62	-8.25	-8.75
4	-3.75	-1.87	-2.50	-3.75	-5.00	-3.37
5	-11.37	-2.62	-7.00	-9.50	-4.50	-7.00
6	-3.50	-2.25	-6.00	-7.25	-4.75	-4.75
7	-3.37	3.50	-5.87	-5.87	-3.37	-3.00
8	-5.37	.25	-9.75	-11.00	-24.75	-10.12
9	-4.62	3.50	6.00	15.37	6.62	+5.37
10	-12.62	-3.25	-8.25	-6.37	-10.75	-8.25
11	-10.25	-4.62	-3.37	-2.75	-8.37	-5.87
Mean	-7.03*	-.27	-4.59*	-4.48*	-5.90*	-4.45*
			CS-			
1	-4.12	18.37	10.25	5.87	-7.25	+4.62
2	-9.25	-11.12	-11.75	-13.00	-14.25	-11.87
3	-20.00	12.50	-7.50	-11.25	-7.50	-6.75
4	-2.37	7.00	3.25	.12	-.50	+1.50
5	-13.87	-1.37	-11.37	-12.62	-8.87	-9.62
6	-16.25	-5.62	-18.12	-13.12	-14.37	-13.50
7	-22.00	-2.62	-14.50	-17.62	-14.50	-14.25
8	-10.12	-1.37	-10.75	-18.87	-28.87	-14.00
9	-3.37	7.25	14.12	17.87	8.50	+8.87
10	-9.87	-3.00	-3.62	-1.75	-7.37	-5.12
11	-11.62	5.25	.25	-2.25	-5.37	-2.75
Mean	-11.17*	+2.30	-4.52	-6.06	-9.13*	-5.72*

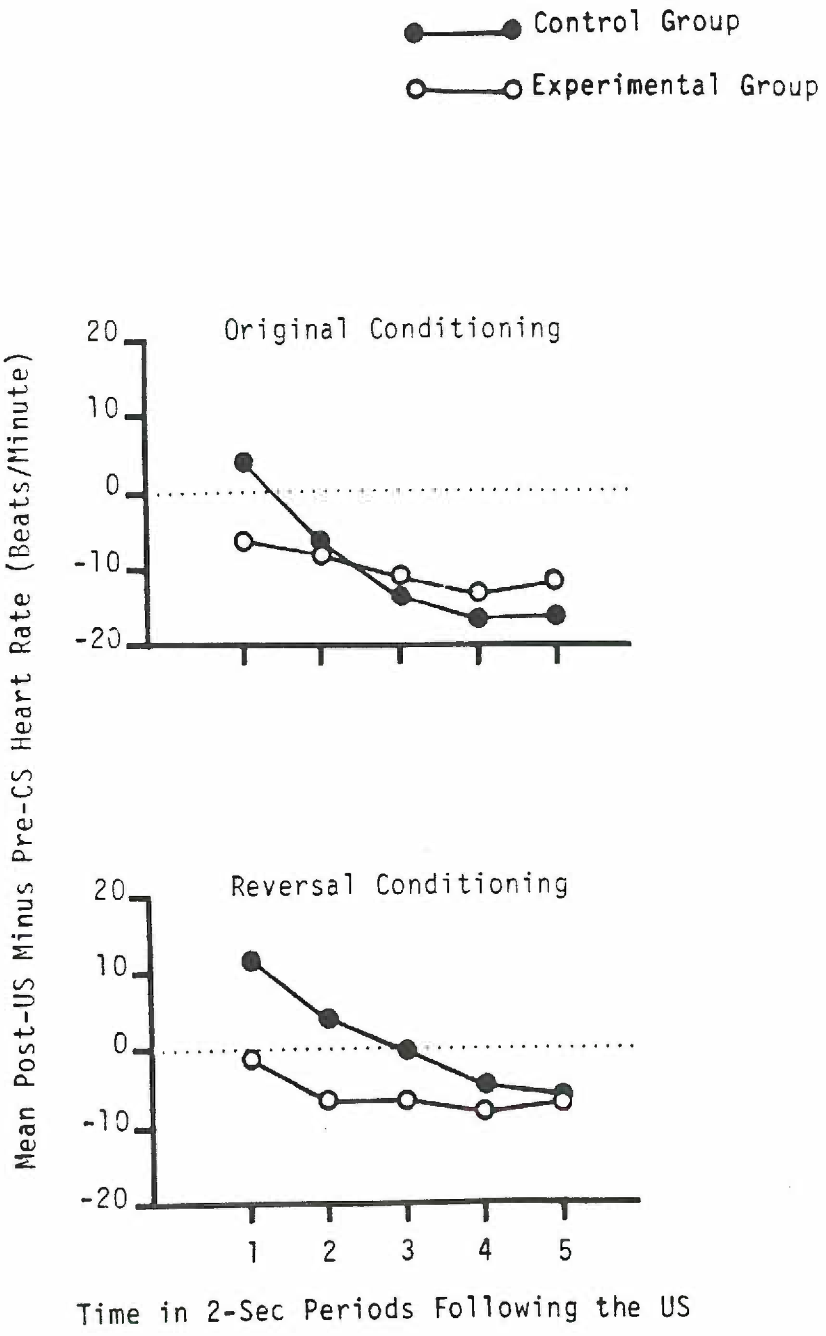
$^{*}p < .05$ when compared to zero.

Unconditioned Heart Rate Responses to the US

Portrayed at the top and bottom of Figure 4 are the unconditioned HR responses of the experimental and control group elicited by the shock US during original conditioning and during reversal conditioning, respectively. Each graph shows the mean HR responses of the groups during successive 2-sec measurement intervals following shock termination averaged over the 4 days of original conditioning and over the 3 days of reversal conditioning. From an inspection of the top of Figure 4, it is clear that the HR unconditioned response of the control group was biphasic containing an accelerative component in the first counting period and decelerative components in the subsequent measurement periods. Maximum HR decelerations occurred in the fourth and fifth counting periods or approximately 8 to 10 sec following shock. Separate $\underline{t}$ tests revealed that the acceleration in the first counting period was significantly different from zero, $\underline{t}(10) = 1.80$, $\underline{p} < .05$, as were the decelerations in the remaining four counting periods (probability at least $\underline{p} < .01$, $\underline{df} = 10$). In contrast to the control group, the unconditioned HR response of the experimental group contained no accelerative component. Instead, the response was monophasic, with decelerations occurring in all counting periods. Like the control group, however, maximum decelerations occurred in the later post-shock measurement intervals.

A groups by counting periods analysis of variance performed on the data in the top half of Figure 4 provided a significant counting periods effect, $\underline{F}(4, 56) = 16.43$, $\underline{p} < .001$, demonstrating that HR changed reliably during the five counting periods. Also significant was a counting periods by groups interaction, $\underline{F}(4, 56) = 5.17$, $\underline{p} < .01$, showing that the HR

Figure 4. Mean pre-CS minus post CS+ and heart-rate responses of the experimental and control groups in five successive 2-sec counting periods following shock averaged over the 4 days of original conditioning and over the 3 days of reversal conditioning. Original conditioning is shown in the top half of the figure and reversal conditioning in the bottom half of the figure.


Control Group
Experimental Group
Original Conditioning
Reversal Conditioning
20
10
0
-10
-20
1
2
3
4
5
Mean Post-US Minus Pre-CS Heart Rate (Beats/Minute)
Time in 2-Sec Periods Following the US

responses of the two groups were reliably different.

In the bottom of Figure 4 are the mean unconditioned reactions of both groups during reversal conditioning. Once again, the control group demonstrated a biphasic HR response characterized by HR accelerations in the first two measurement intervals, and HR decelerations in the fourth and fifth measurement periods. Separate *t* tests showed that the first, $t(10) = 7.21$, $p < .005$; fourth, $t(10) = 2.04$, $p < .05$, and fifth counting periods, $t(10) = 2.73$, $p < .025$, were significantly different from zero, establishing the reliability of the biphasic response pattern. As was true of original conditioning, the HR reaction produced by shock in the experimental group did not contain an accelerative component. Thus, HR was below baseline in this group in each of the five post-shock counting periods, although the magnitude of the decrease in the first counting period was somewhat smaller than those in succeeding periods. A groups by counting periods analysis of variance, carried out on the reversal conditioning data, demonstrated a reliable difference in the overall magnitudes of the HR responses of the groups, $F(1, 14) = 4.98$, $p < .05$. There was also a significant counting periods effect, $F(4, 56) = 4.15$, $p < .01$, and a significant groups by counting periods interaction, $F(4, 56) = 4.08$, $p < .01$, indicating that HR changed reliably following shock and that the HR change was different for the two groups.

Summaries of the main findings relating to the unconditioned HR responses of the two groups are provided in Tables 5 and 6. From an examination of Table 5, it is evident that during each phase of conditioning, only one of the experimental dogs failed to display decelerative HR changes to shock in each of the five post-shock measurement periods. The

Table 5

Mean HR responses of the experimental animals during 5 successive 2-second intervals following shock termination.

Subject Number	Counting Periods 1	2	3	4	5	Mean
			Original Conditioning			
1	-4.31	-3.37	-8.06	-10.40	-9.93	-7.21
2	-22.68	-24.09	-22.68	-26.43	-23.62	-23.90
3	1.96	5.71	1.50	-1.31	-1.31	+1.31
4	-1.31	-7.40	-13.50	-10.68	-9.75	-8.53
5	-7.21	-8.15	-11.90	-14.25	-15.65	-11.43
Mean	-6.71	-7.46	-10.93*	-12.61*	-12.05*	-9.95*
			Reversal Conditioning			
1	4.50	.12	-3.00	.75	-3.62	-.25
2	-.62	-11.87	-8.75	-5.00	-8.12	-6.87
3	-.87	-2.12	-2.12	-7.75	-7.12	-4.00
4	-3.50	-9.75	-8.50	-10.37	-7.87	-8.00
5	-7.62	-12.00	-13.25	-15.75	-10.12	-11.75
Mean	-1.62	-7.12*	-7.12*	-7.62*	-7.37*	-6.17*

*p < .05 when compared to zero.

Table 6

Mean HR responses of the control animals during 5 successive 2-second intervals following shock termination.

Subject Number	Counting Periods 1	2	3	4	5	Mean
			Original Conditioning			
1	8.06	-.84	-7.40	-12.09	-11.15	-4.68
2	6.75	-8.25	-12.93	-14.34	-16.21	-9.00
3	5.06	-9.93	-22.12	-27.28	-26.34	-16.12
4	15.28	-1.59	-11.43	-21.28	-23.62	-8.53
5	9.84	-11.25	-19.68	-23.43	-25.78	-14.06
6	10.40	-6.46	-11.15	-19.59	-19.12	-9.18
7	9.37	1.40	-7.03	-15.93	-13.59	-5.16
8	-13.12	-16.87	-10.31	-9.84	-5.62	-11.15
9	-2.71	-10.68	-10.21	-6.93	-6.46	-7.40
10	.65	-3.09	-13.40	-15.75	-17.62	-9.84
11	-1.68	-9.65	-17.62	-15.75	-15.28	-12.00
Mean	+4.36*	-7.02*	-13.03*	-16.57*	-16.44*	-9.74*
			Reversal Conditioning			
1	10.00	9.37	8.12	-1.87	-1.25	+4.87
2	22.62	5.75	8.25	.12	6.37	+8.62
3	9.87	6.75	4.25	-2.62	-10.75	+1.50
4	10.00	9.37	9.37	1.25	0.0	+6.00
5	9.87	-6.37	-13.87	-18.87	-19.50	-9.75
6	4.62	-14.75	-14.12	-12.25	-4.12	-8.12
7	14.12	1.62	-14.62	-11.50	-12.75	-4.63
8	4.62	5.87	5.25	9.00	.87	+5.12
9	17.87	11.00	11.62	.37	-5.25	+7.12
10	10.50	3.62	-3.87	-8.87	-12.00	-2.12
11	11.62	7.87	-2.12	-7.75	-9.62	+0.00
Mean	+11.43*	+3.65	-.16	-4.82*	-6.18*	+.78

*$p < .05$ when compared to zero.

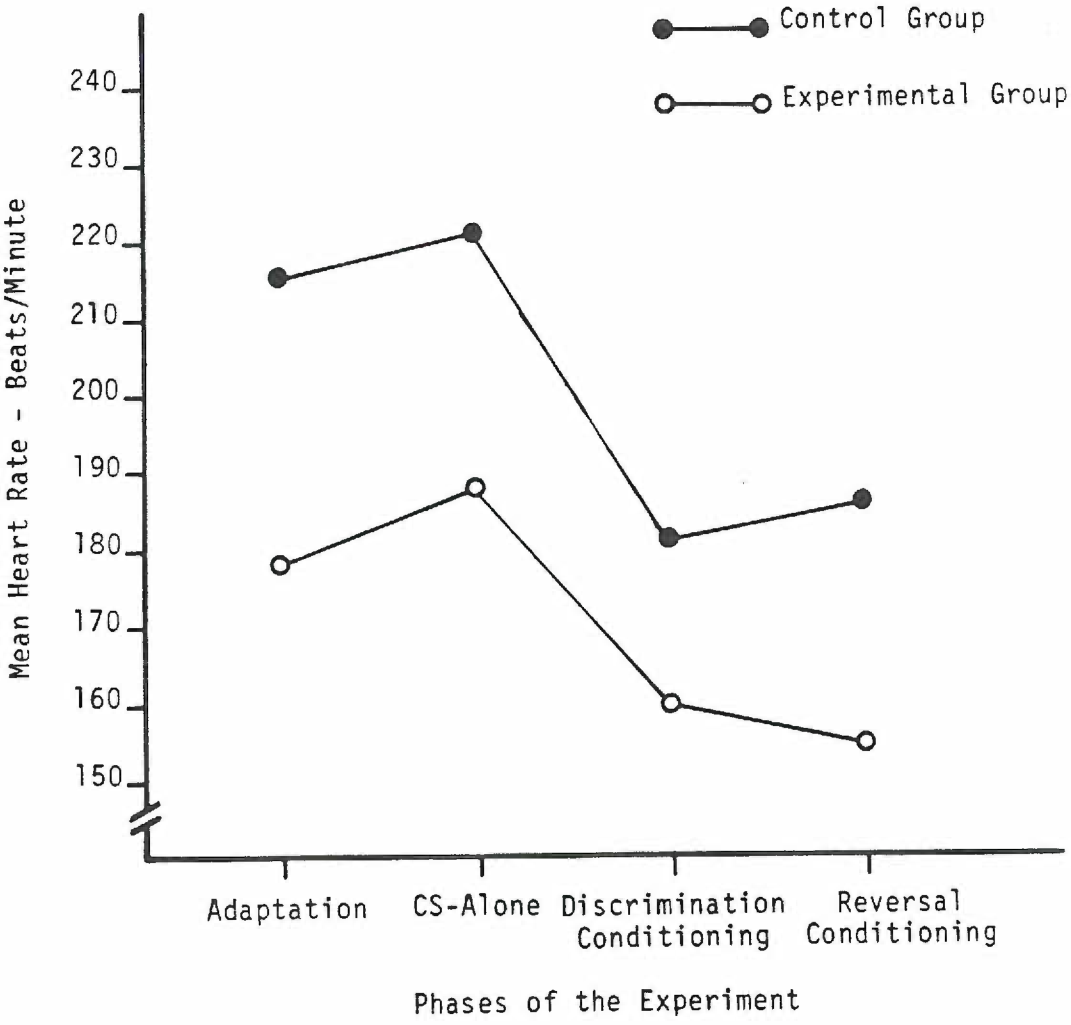
magnitudes of the decelerations occurring in Dogs 2 and 5 were particularly large. In neither phase were the HR changes in the first counting period significantly different from zero, but in Counting Periods 3, 4, and 5 significance was achieved. Table 6 indicates that HR acceleration in the first counting period of original conditioning was shared by 8 animals in the control group and by all 11 animals during reversal conditioning. In both cases, the group means were significantly different from zero. In several control animals, HR accelerations persisted into the second counting period and occasionally even into the third period.

Baseline Heart Rate during the Four Phases of the Experiment

Depicted in Figure 5 are the mean baseline HRs of the experimental and control groups averaged over the pre-CS+ and pre-CS- periods during the CS-alone, discrimination conditioning, and reversal conditioning phases of the study and over the 10-sec periods of the adaptation phase. This figure shows that baseline HR of the experimental group was consistently below that of the control group during each phase of the experiment and that the difference between the groups was approximately the same during each phase. The figure also shows that both groups had lower baseline HRs during the discrimination and reversal conditioning phases than during the adaptation and CS-alone phases.

A 2 by 4 analysis of variance (groups by experimental phase) provided a significant groups effect, $\underline{F}(1, 14) = 15.04$, $\underline{p} < .005$, confirming the reliability of the overall difference in the baseline HR of the experimental and control groups. There was also a reliable experimental phase effect, $\underline{F}(3, 42) = 29.17$, $\underline{p} < .001$, establishing that baseline HR of the

Figure 5. Mean baseline HR of the experimental and control groups in the four phases of the experiment.

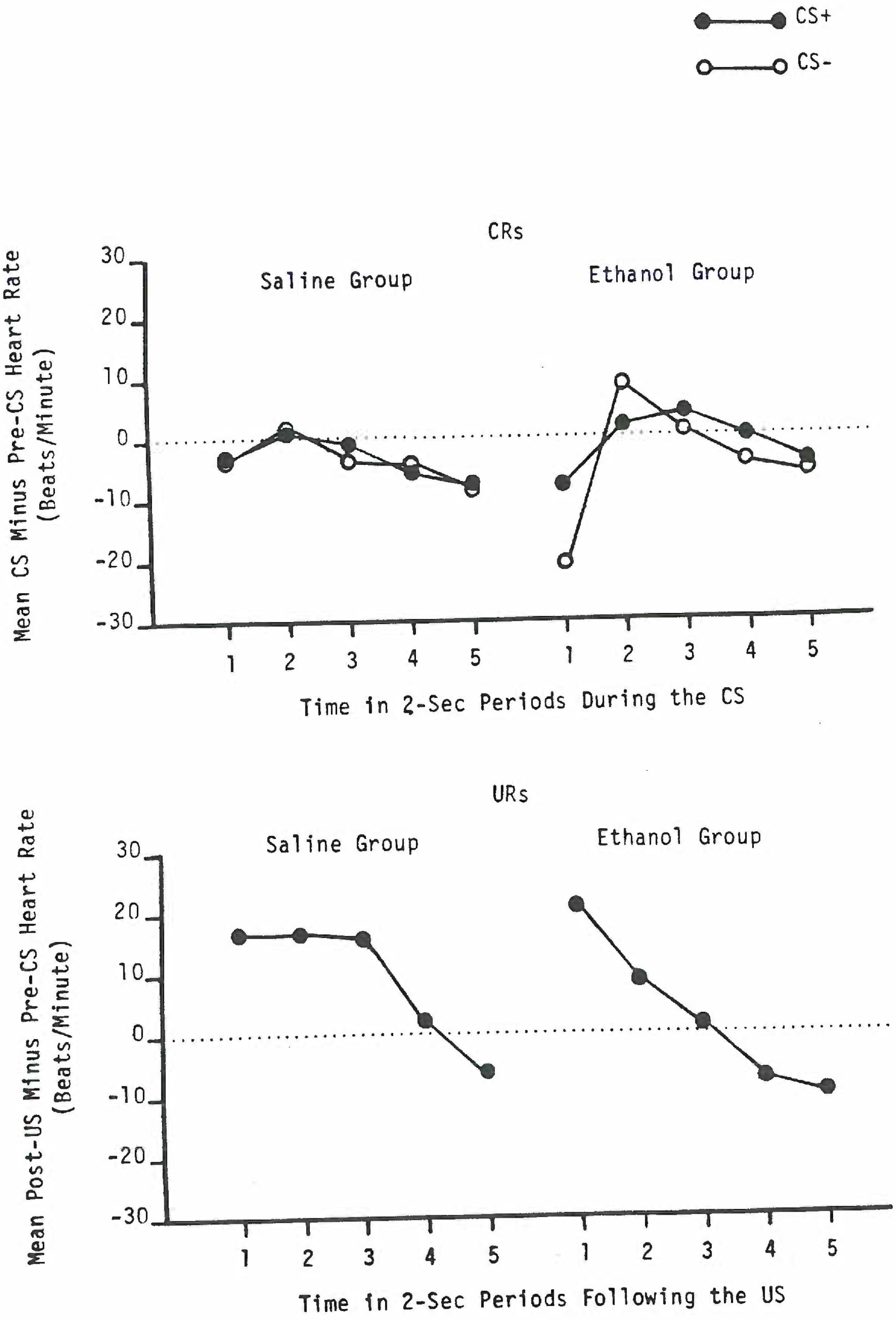

Control Group
Experimental Group
Mean Heart Rate - Beats/Minute
240
230
220
210
200
190
180
170
160
150
Adaptation
CS-Alone
Discrimination Conditioning
Reversal Conditioning
Phases of the Experiment

groups changed reliably across the four phases. A subsequent Newman-Keuls test showed that each group's baseline HR in the adaptation and CS-alone phases was reliably higher than those in the discrimination and reversal phases. There were no reliable differences between adaptation and CS-alone or between discrimination and reversal conditioning.

Effects of Ethanol on Reversal Conditioning

On the fourth day of reversal conditioning, seven control animals received an i.v. injection of 1.5 g/kg of ethanol (30% v/v) 10 minutes prior to the beginning of conditioning, and the four remaining control animals were given an equal volume of saline. The mean HR responses of the two groups in 2-sec periods of CS+ and CS- averaged over the 16 trials with each CS are shown at the top of Figure 6. This part of the figure reveals that the HR reactions of the ethanol group were larger than those of the saline group. This was especially true of the early HR changes to CS-, with the ethanol group showing HR deceleration in the first counting period and HR acceleration in the second counting period that matched in terms of magnitude those HR changes that occurred to CS- during most of original conditioning and during the first day of reversal conditioning. A similar recovery of the HR response to the CS+ in the ethanol group was not evident. A three-way (drug treatment by counting periods by type of CS) analysis of variance provided a significant counting periods effect, $\underline{F}(4, 36) = 9.62$, $\underline{p} < .001$, and a significant drug treatment by counting periods interaction, $\underline{F}(4, 36) = 4.74$, $\underline{p} < .005$, indicating that the HR response patterns of the two groups were reliably different. A Newman-Keuls test showed that the mean HR responses of the

Figure 6. At the top of this figure are the mean CS minus pre-CS heart rate responses of the saline and ethanol groups in 2-sec periods of CS+ and CS- averaged over 16 trials. In the lower half of the figure are the mean post US minus pre-CS heart rate responses of both groups following shock termination averaged over 16 trials.


CS+
CS-
CRs
Saline Group
Ethanol Group
Mean CS Minus Pre-CS Heart Rate (Beats/Minute)
30
20
10
0
-10
-20
-30
1
2
3
4
5
1
2
3
4
5
Time in 2-Sec Periods During the CS
URs
Saline Group
Ethanol Group
Mean Post-US Minus Pre-CS Heart Rate (Beats/Minute)
30
20
10
0
-10
-20
-30
1
2
3
4
5
1
2
3
4
5
Time in 2-Sec Periods Following the US

two groups to CS+ and CS- were significantly different from each other in the first counting period but not in the remaining counting periods. To make sure that the ethanol and saline groups were not different from each other prior to the administration of ethanol, the HR responses of the two groups were compared on Day 3 of reversal conditioning using the same type of analysis of variance that was employed on Day 4. This test provided no significant outcomes.

At the bottom of Figure 6 are the unconditioned HR responses of the saline and ethanol groups to the shock US in 2-sec intervals following shock termination averaged over the 16 trials. In contrast to the responses to the CSs this part of the figure indicates that ethanol did not change the HR response to shocks. Both groups responded with large accelerative HR changes in the earlier counting periods and smaller decelerative changes in later periods. A groups by counting periods analysis of variance revealed a significant counting periods effect, $\underline{F}(4, 36) = 13.52$, $\underline{p} < .001$, demonstrating that the change in HR during the counting periods was reliable.

DISCUSSION

There were six major findings in the present experiment. First, during the preconditioning CS-alone phase, the experimental and control groups showed similar decelerative HR responses to the CSs. In neither group did the responses appear to habituate over trials. Second, the experimental and control groups demonstrated distinctively different HR responses to the CSs during the original discrimination conditioning phase. Within this phase, the experimental group responded with a monophasic HR deceleration to both CS+ and CS-. Moreover, there was no evidence of differential responding to the two CSs in the experimental group. In contrast, the control group's HR responses to both CSs during original conditioning were basically triphasic in nature. In the first counting period of the CSs, HR decelerated. This was followed by HR acceleration in the second counting period and then by HR deceleration in the remaining counting periods. During this phase there was some evidence of differential responding to the reinforced CS (CS+) and unreinforced CS (CS-). Third, no major changes were found in the HR responses of the experimental group to CS+ and CS- during reversal conditioning. Both CSs continued to elicit monophasic decelerations similar to those seen during original conditioning. On the other hand, the HR responses of the control group changed during reversal conditioning. This change was seen as an attenuation of the HR decelerations and accelerations in the first and second counting periods, respectively, of both CSs. Fourth, the unconditioned HR responses of the two groups to the shock US were different from each other. The experimental group demonstrated only HR decelerations, while the control group displayed HR acceleration shortly after shock, followed by HR deceleration. Fifth, baselevel HR in the experimental group was significantly lower than that of the control group in each phase of the study. The magnitude of the difference between the groups was approximately the same in each phase. Sixth, administration of ethanol enhanced the HR decelerations of the control group that were present at the beginning of both CS+ and CS-. In fact, ethanol appeared to result in the immediate return of the triphasic decelerative-accelerative-decelerative HR reaction that had diminished over the course of reversal conditioning.

Preconditioning CS-alone

Upon their initial application, low to moderate intensity CSs typically evoke observable responses that seem to signify that the CS has been noticed. Dogs typically demonstrate a number of skeletal motor responses to auditory CSs ranging from perking up the ears, head turning, and postural adjustment, as well as autonomic responses reflected in changes in HR, blood pressure, and respiration. Pavlov called this response complex the orienting reflex, the what-is-it reflex, or the investigatory reflex. At the present time, these kinds of initial reactions to novel CSs are generally referred to as the orienting response (OR) (Sokolov, 1963).

It has been suggested that the OR may be useful in evaluating the physiological and behavioral development of young organisms. Clifton (1974b) proposed that the direction of the heart rate component of the OR was a good index of cortical development in the human infant. Pavlov (1927) originally emphasized the role of the OR in assessing the degree of maturation of adaptive behaviors. He felt that on a continuum of adaptive behaviors, the OR may be the first response that can be measured and that it occurred prior to the time that organisms are capable of forming a CR. Pavlov also believed that if the original ORs were too vigorous, they could interfere with the formation of the CR. To alleviate this problem, a number of trials with the CS alone are typically given to habituate the OR before conditioning begins.

In the present experiment, the OR in both the experimental and control groups was a monophasic HR deceleration that did not differ in magnitude or form to the two different tone CSs. There was no evidence that OR magnitude diminished over the CS-alone trials that were given each group. The fact that the control group showed significant decelerations to both tones, while the experimental group demonstrated significant HR decreases to only one CS, suggests that the control group may have been more reactive than the experimental group.

In contrast to the decelerative ORs observed in the present study, other experiments have shown that the direction of the HR OR of adult dogs to auditory stimulation is generally an acceleration (e.g., Fitzgerald, 1966; Lynch, 1967). In addition, Fuller and Christake (1959) reported that an air puff of moderate intensity delivered to the back of the head of dogs 20 to 30 days old produced cardioaccelerative ORs. The HR responses of dogs older than 30 days varied between deceleration and acceleration. Freedman, King, and Elliot (1961) have demonstrated that the HR responses in young dogs to novel stimuli such as tones, voices, and shocks depended upon the age and the history of "socialization" of the animals. These authors reported the magnitude but not the direction of the changes, making it difficult to relate their findings to the present study.

Volokhov (1968) suggested that in immature organisms the cardiac OR is generally a slowing of the heart, but that the response could vary according to the species being examined and the modality of the eliciting stimulus. Conceivably, the reason the dogs in the current experiment failed to show accelerative HR ORs comparable to those seen in adult dogs was because the neural centers controlling their cardiovascular activity were not fully developed. The most dramatic changes in the development of CNS functioning in dogs have been shown to occur within the first 6 weeks following birth (Fox, 1971). Beyond that point, further development occurs at a less rapid rate. It will be recalled that the dogs in the current experiment were approximately 6 weeks old at the start of training and thus may have been within the age range when cardiac control centers were still changing rapidly.

Original Discrimination Conditioning

According to Pavlov (1927), discrimination or differential classical conditioning involved the acquisition of a primary CR to the reinforced CS+ and the attachment of a generalized CR to the nonreinforced CS-. It was thought that the generalized CR gradually extinguished through nonreinforcement. Differential responding was regarded by Pavlov as more difficult to master than the formation of a primary CR. This was due to the fact that the organism had to learn two things: first to respond to the CS+ and second to inhibit responding to the CS-. The results of several studies have indicated that differential responding to CS+ and CS- in a classical conditioning paradigm may be especially difficult for young organisms to master (Kasatkin & Levikova, 1935b; Naito & Lipsitt, 1969; Ovakimyan, 1961; Volokhov, 1968).

Finding that the HR responses of the experimental group to the CS+ and CS- during original discrimination conditioning were not significantly different from each other is in general agreement with these other results. In the present case, the HR reactions of the experimental group to both CSs were decelerative in direction, and their magnitudes were similar to those occurring to the CSs during the preceding CS-alone phase. The similarity between the original HR ORs and the HR responses during discrimination training adds support to the view that little, if any, HR conditioning occurred in this group. On the other hand, it is possible that some conditioning did in fact occur, but that it was not reflected in performance, perhaps because of the immaturity of the experimental group's cardiovascular system. Along these same lines, the absence of accelerative HR changes to the CSs in the experimental group like those that were shown by the control group suggests that the cardiac regulatory systems of the two groups may not have been the same. This hypothesis will be explored in more detail in a subsequent section.

While it is true that the control group showed evidence of discrimination conditioning in the form of differential HR responding to CS+ and CS- on the original discrimination trials, the overall magnitude of the response differences was extremely small. This suggests that there was considerable generalization of the HR response from CS+ to CS-.

Generalization of cardiac responses is also known to occur in discrimination studies involving adult dogs. In a study by Lynch (1966), nine adult dogs showed a generalized cardiac reaction, usually an acceleration, to the CS- throughout most of a discrimination training period. Although a criterion for discriminated leg-flexion responding was achieved after an average of 100 trials with each tone CS (480 and 630 Hz) only three of the dogs showed larger magnitude HR responses to the CS+ rather than CS-. Kakigi (1971) reported similar results. He used five tones (600, 800, 1000, 1200, and 1400 Hz), only one (1000 Hz) of which was followed by shock. Eight adult dogs were given five trials with each CS on 21 consecutive days. Initially a cardioacceleration was produced by all of the CSs. By the final 3 days of training, moderate HR accelerations still occurred to all CSs with the magnitude of the accelerations being larger to the CS+. These findings along with those reported by Lynch (1966), indicate that a relatively large number of trials may be necessary for adult dogs to demonstrate differential cardiac responding in a discrimination paradigm. Even then, the magnitude of the discrimination may not be very large.

Evidence that young dogs also have difficulty forming successful discrimination was provided by Ovakimyan (1961). He observed that 3 to 4-month-old dogs developed a conditioned vascular response to a CS+ as rapidly as that seen in adult dogs. This occurred after approximately 40 to 50 paired CS-US presentations. However, differential responding between CS+ and CS- was not established in the young dogs in that the CR attached to CS+ tended to generalize to CS-, even after approximately 30 nonreinforced presentations of the CS-.

Volokhov (1968) reported comparable difficulties in a study of leg-flexion conditioning in young dogs. He found that the dogs initially showed nonspecific motor activity to CS+ and to CS-. There was then a period of variable responding to both CSs. By the end of the second month of life, and after an unspecified number of trials, the dogs demonstrated differentiation by giving leg flexions to CS+ and not to CS-. This is one of the few studies in which successful differentiation was obtained in dogs as young as 2 months of age. In no experiments including the present one has convincing evidence of discrimination learning been found in young dogs using cardiovascular reactions as an index of learning. While inhibition of motor activity to a CS- appears difficult for young dogs to achieve, it appears even more difficult when autonomically mediated reactions are involved.

Reversal Discrimination Conditioning

During reversal conditioning, the reinforcement contingencies in effect during original discrimination conditioning were changed, such that the original CS+ was now presented without the US, while the previous CS- was now paired with the US. It can be argued on the basis of several accounts of discrimination conditioning (Mackintosh, 1974) that reversal conditioning involves the extinction of excitatory and inhibitory tendencies built up to the previous CS+ and CS-, coupled with the acquisition of like tendencies to the new CSs. This would lead to the expectation that the HR responses developed to CS+ and CS- during original discrimination conditioning would weaken and then eventually reappear to the respective CSs during reversal conditioning.

The differences between the experimental and control groups that were present during original conditioning gradually diminished over the course of reversal training. This was due to changes in the performance of the control group, rather than to changes in the performance of the experimental group. It was found that the HR responses of the experimental group during reversal conditioning were nearly identical in form and magnitude to those occurring during original conditioning. In both cases, the HR responses to the 1500 Hz tone were slightly smaller HR decelerations than those given to the 700 Hz tone. The same type of responding also existed in the CS-alone phase. In brief, the experimental group tended to respond to the CSs in the same way throughout the experiment, even when one of the CSs was paired with shock.

The HR responses of the control group were gradually modified during reversal conditioning. By the end of the third day of the reversal procedure, the responses to both CSs had changed from triphasic deceleration-acceleration-decelerations to monophasic decelerations. The loss of the accelerative component to both CSs is difficult to understand. Conceivably, the disappearance was somehow related to the fact that the animals had already received extensive conditioning training before the shift to the reversal procedure was made. Pavlov (1927) reported that conditioned salivary responses in dogs frequently decreased in magnitude and occasionally were lost after a large number of conditioning trials even though the animals continued to received food or acid reinforcement. He explained the decrement on the basis of an inhibitory process that was generated by repeated reinforcement. If a comparable type of inhibition had been operating in the current study the decrement in the control group's accelerative reaction would have occurred even if the relationships between the CSs and the US had not been reversed.

A second possibility that could help account for the reduction in the responding of the control group is that the shock US may have lost some of its aversiveness over the course of conditioning. It is known that the effects of electric shock can habituate with repeated shock exposure (e.g., MacDonald, 1946). However, in the present experiment this seems unlikely, as the magnitude of the unconditioned HR acceleration produced by shock was actually slightly larger during reversal conditioning than during original conditioning. Also, there was no change in the overall magnitude of the HR reaction triggered by the shock US in the experimental group.

A final reason for the decrease in the control group's responding during reversal may be extinction. Conceivably, when the US no longer followed the original CS+, extinction of the accelerative HR reaction took place. As was mentioned previously, this may be part of the normal process in reversal conditioning (Mackintosh, 1974). In this context, it is possible that had more training trials been given, the triphasic decelerative-accelerative-decelerative HR response might have reappeared during the reversal phase.

Effects of Ethanol

The administration of ethanol on the last day of reversal conditioning altered the HR responses of the ethanol group to both CSs. Basically, the magnitude and form of the HR response to the CS- (the original CS+) resembled that seen during original discrimination training (i.e., deceleration-acceleration-deceleration) and during early reversal training. Although the HR response to the CS+ also changed in the ethanol group, this change was not as conspicuous as that to the CS-.

Previous investigations of the effects of ethanol on discrimination learning in dogs have suggested that ethanol "disinhibits" or "releases from suppression" certain behaviors. Nikiforovski (1910, cited in Andreyev, 1934) reported that after a discrimination had been formed in adult dogs, low doses of ethanol did not influence responding to the CS+, but did increase responding to the CS-. Andreyev (1934), using a single dog, also reported that low doses of ethanol disrupted an established discrimination by increasing responding to the CS-. Finally, both Gantt (1935) and Dworkin, Bourne, and Raginsky (1937) demonstrated what they felt were "disinhibitory" effects of ethanol on the performance of dogs. The disruption that they observed principally involved an increase in responding to the CS- after a reasonably stable level of discrimination had been shown.

In general, then, the recovery of the triphasic HR response of the group given ethanol in the current experiment is consistent with the view that ethanol has the capacity to disinhibit suppressed responding. While it may not be possible to specify the mechanism that was responsible for the HR suppression itself, the HR reaction was clearly augmented or reinstated following the administration of ethanol. Moreover, the fact that the recovery of the triphasic responses in more or less its original character suggests a degree of permanency normally attributed to learned reactions.

Unconditioned Responses

The unconditioned responses of the experimental and control groups to the shock US were not the same. The experimental group responded to shock during the original and reversal discrimination phases of the study with a monophasic deceleration that tended to increase in magnitude over the 10-sec post-shock measurement interval. Thus, as was true of the HR responses to the CSs on the CS-alone, original discrimination, and reversal trials, the experimental group gave no sign of an accelerative HR change, even to shock. Inspection of the records of individual experimental dogs revealed only a few instances of HR accelerations, and these were relatively small in magnitude.

In contrast, the unconditioned HR responses of the control group during both original and reversal discrimination conditioning were biphasic, consisting first of an acceleration followed by a deceleration. In the original discrimination phase, 8 of the 11 dogs demonstrated this biphasic pattern, while in the reversal discrimination phase, each of the 11 dogs responded in a biphasic fashion. Generally, the HR acceleration was relatively short in duration lasting 2 to 4 sec, whereas the HR deceleration persisted well over 6 sec.

Fuller and Christake (1959) found that the HR responses of 20 to 50-day-old dogs to painful shock were cardioaccelerations. In most cases, the magnitude of the HR acceleration was approximately 20 bpm, which is close to what the control group showed in the present study. Although Fuller and Christake did not report finding a decelerative component to the unconditioned reaction like that observed here, they did not specify at what time HR was measured with respect to shock. Therefore, it is possible that relatively long latency HR decreases were present but were not recorded.

In adult dogs, the HR response to electric shock has generally been a consistent cardioacceleration, although instances of cardiodecelerations have been noted (Antal & Gantt, 1970; Dykman, 1960; Dykman, Mack, & Ackerman, 1965; Katcher, Solomon, Turner, LoLordo, Overmier, & Rescorla, 1969; Mack, Davenport, & Dykman, 1961). Katcher et al. (1969) examined the relative contributions of sympathetic and parasympathetic influences on HR responses to electric shock in adult dogs paralyzed with d-tubocurarine chloride. They reported that HR acceleration to shock was diminished or absent in dogs that were sympathectomized by excision of the sympathetic chain and ganglia from T_7 or T_8 to and including the stellate ganglion. This suggested that the accelerations were mainly due to increased sympathetic outflow. On the other hand, the fact that some accelerations occasionally occurred suggested that the vagal system also participated in the response, but its contribution was thought to be relatively minor. On the basis of these findings, it can be conjectured that the reason the experimental group did not show accelerative HR changes to the shock may have been that the dogs lacked appropriate sympathetic innervation. Conceivably, the consistent HR deceleration to shock in the experimental group is an indication that cardiac control centers in the experimental and control groups were not equally developed.

Baselevel Heart Rate

During each of the four phases of the study, baseline HR in the experimental group was lower than that of the control group. The mean levels for the experimental group during the adaptation, CS-alone, original discrimination, and reversal discrimination phases were 178 bpm, 188 bpm, 159 bpm, and 154 bpm, respectively. For the control group they were

215 bpm, 221 bpm, 180 bpm, and 186 bpm, respectively. The basal HR of unanesthetized resting dogs has been reported to range from 50 to 160 bpm (Gasser & Meek, 1914; Horwitz, Spanier, & Wiggers, 1953; Long, Truex, Friedmann, Olsen, & Phillips, 1958; Murphy, 1942; Samaan, 1934a, 1934b). A value of 100 bpm is often mentioned as being representative. Neural control of basal HR in adult dogs has been found to be primarily parasympathetic in nature (Essex, Herrick, Baldes, & Mann, 1943; Essex, Herrick, Mann, & Baldes, 1943; Long et al., 1958; Murphy, 1942; Samaan, 1934a, 1934b).

From a developmental standpoint, the experimental evidence suggests that parasympathetic control of canine HR is relatively weak at birth (Fox, 1965; Kellogg, 1927; Truex, Scott, Long, & Smythe, 1955). As might be expected, from incomplete parasympathetic development and function, baseline HR of young dogs (e.g., under 4 months of age) is generally higher than that of adult dogs. Truex et al. (1955) demonstrated in unanesthetized dogs, that there was nearly a linear decrease in baseline HR from birth to 80 days of age. The average HR at birth was 240 bpm, at 5 to 6 weeks of age 200 bpm, and at 10 weeks of age 150 bpm. More recently, both Scott (1963) and Fox (1966) reported that the resting HR of 1 to 5-week-old dogs increased slightly during the first week after birth and then decreased sharply over the following weeks. Newton, Murphree, and Dykman (1970) found that the mean HR of 2 to 6-week-old dogs was around 210 bpm. This value was close to that reported by Truex et al. (1955) for dogs of this age, and to that observed in the control dogs in the present experiment.

Truex et al. (1955) reported electrophysiological and histological findings related to the development of cardiac control in young dogs. They noted that stimulation of either vagus nerve caused cardiodeceleration. When bilateral vagotomy was performed, no increase in HR was obtained indicating an absence of vagal tone. Histologically, it appeared that while the preganglionic fibers of the vagus nerve reached the heart, most of the postganglionic intracardiac cells were undifferentiated and lacked stainable processes. It was thought that the few postganglionic vagal neurons that were differentiated were responsible for the HR deceleration that was seen following vagal stimulation. On the other hand, even at birth, fine myocardial and vascular nerves were abundent and presumed to be sympathetic in origin. Truex et al. (1955) concluded that in dogs, functioning postganglionic sympathetic fibers reached the myocardium before postganglionic parasympathetic neural elements were fully developed.

Pharmacological evidence also supports the contention that parasympathetic cardiac controls are not well developed at birth in dogs. As early as 1880 Anrep (cited in Truex et al., 1955) reported that atropine (a parasympathetic blocker) had little or no effect on HR in newborn dogs. Lhota (1911) also came to the same conclusion. In a more recent study, Fox (1965) found a progressive increase in the effects of atropine on HR as the dogs grew older suggesting increasing parasympathetic regulation.

Several factors are known to influence the normal development of basal HR levels in dogs. The works of Dykman, Murphree, and Peters (1969) and Newton et al. (1970) have demonstrated the important role that constitutional (genetic) factors play in the determination of the canine basal HR. In the Newton et al. (1970) study, the offspring of two strains of pointer dogs, one bred for nervousness (pronounced freezing and withdrawal behavior) and the other for "normal" behavior, were examined for incidence of AV block and for differences in basal HR at 2 to 6 weeks of age. The "normal" group had a mean basal HR of 210 bpm, which was similar to the values of the control group in the current investigation. The group bred for nervous behavior had a mean basal HR of 183 bpm which was similar to the HR values of the experimental group in the present study. Interestingly, these early HR differences persisted in the adult dog, even though the overall rates of both groups decreased (Dykman et al., 1969). It should be emphasized that careful repeated inbreeding was necessary to establish these differences, a procedure which obviously was not employed in the present experiment.

Recent evidence also illustrates that postnatal experiences can be a factor influencing the development of autonomic cardiac regulation (Fox, 1966; Hofer, 1974; Scott, 1963). Fox (1966) compared the HR of an experimental group of dogs that received 1 hr of special stimulation each day from birth for 5 weeks with that of an unhandled control group. The resting HR of the control group increased from 200 to 230 bpm during the first week of life. During the following 4 weeks, the HR of this group declined to about 160 bpm. In contrast, the handled group showed a stable HR level around 200 bpm during the 5 weeks of the study.

In a later report involving the same dogs, Fox (1971) noted that in general, the handled dogs readily engaged in exploratory behavior, were very sociable, were dominant in play with their peers, vocalized frequently, and mastered a barrier test problem quickly. On the other hand, the nonhandled control dogs were subordinate in play with their peers, were highly emotional in novel environments, and were slower to master the barrier problem. The author suggested that the heightened emotionality of the nonhandled group may have interfered with their performance.

Differences in emotionality between the experimental and control groups also seemed to be present in the current experiment. The control dogs also seemed more emotional than experimentals, frequently whining and barking in the test chamber. The experimental dogs were generally quiet in the test chamber and easier to prepare for the test sessions. However, it is important to stress that every effort was made in the present study to insure that all of the dogs were treated identically as far as handling was concerned. Thus, it seems likely that factors other than handling are needed to explain the group differences in baseline HR and emotionality.

One possible mechanism that may account for the lower baseline HR in the experimental group as compared to the control group could be that prolonged intrauterine ischemia prevented normal development of cardiac regulatory processes. In view of the fact that parasympathetic control of HR is not well established in normal young dogs (Fox, 1966; Kellogg, 1927; Truex et al., 1955), it does not seem likely that a further retardation in this system would change baselevel HR appreciably. However, this may not be true for the sympathetic system, as this system appears to contribute importantly to HR regulation in young dogs. Thus, if the development of the sympathetic system was retarded, this could have caused a decrease in basal HR like that observed in the experimental dogs. In short, this proposition suggests that the experimental dogs were more immature at birth, particularly in terms of normal neurological innervation of the heart.

Both the experimental and control groups demonstrated a decline in baseline HR beginning with the original discrimination phase. Similar decreases in baseline HR have also been shown in rats receiving aversive classical conditioning training (Fitzgerald & Martin, 1971). Conceivably, the decreases in the present case represented a general habituation process to the experimental environment.

One final comment that should be made regarding baselevel HR is that differences in baselevel HR between the experimental and control groups could contribute to the HR response differences to the CS and US observed during discrimination and reversal conditioning. For example, according to the Law of Initial Values (Wilder, 1967), the higher the baselevel, the more difficult it is to obtain increases in activity. With respect to the present experiment, this hypothesis would suggest that HR accelerations would be more likely to occur in the experimental group than in the control group because baselevel HR was lower in the experimental group than in the control group. However, just the opposite occurred in the current experiment. Nevertheless, the Law of Initial Values illustrates the potential importance of baselevel responding in modifying autonomic responses.

Behavioral Sequalae of Insufficient O_2

Several investigations have shown behavioral effects following prenatal O_2 deprivation (Bunch, 1952; Martin & Becker, 1971; McCullough & Blackman, 1974; Meier, 1958; Meier, Bunch, Nolan, & Scheidler, 1960;

Meier & Menzle, 1955; Vierck, King, & Ferm, 1966; Vierck & Meier, 1963). In general, the hypoxic offspring in these studies were inferior to controls on various measures of performance. In the current investigation, there was little evidence to suggest that conditioned HR responses were established in the experimental group. On the other hand, discriminated HR changes, albiet small, were demonstrated in the control dogs. In this sense, the experimental dogs appeared inferior in their ability to learn.

Several of the investigations concerning prenatal (Vierck & Meier, 1963; Vierck, King, & Ferm, 1966) and neonatal (Becker, 1958; Saxon, 1961a, 1961b) oxygen deprivation have reported hypoemotionality among the treated animals. Measures of emotionality in these studies included observation of behavior in an open field, reaction to electric shock, reaction to handling and prodding, and excretion rates under stress. As noted earlier, it was the subjective opinion of the people handling the dogs in the present investigation that the experimental animals were also less emotional and more lethargic than the control animals. Control dogs in the current experiment whined and barked more in the experimental chamber, particularly in response to shock, than did the experimental dogs which were generally quiet in the test chamber.

In most of the previous studies using mammals, the procedures that were used to produce oxygen deprivation to the fetus were known to render the mother hypoxic as well. Oxygen deprivation in the pregnant mammal produces many physiological reactions that may potentially affect the fetus, only one of which may be O_2 deprivation. In the present experiment, the intrauterine technique that was used was developed by Hodgkinson, Hodari, and Bumpus (1967), and it involved mechanical restriction of uterine blood flow. Although other nutrients in addition to O_2 may have been reduced by this procedure, O_2 reduction was felt to be the most critical factor changed.

SUMMARY AND CONCLUSIONS

The purpose of the present investigation was to compare the classically conditioned HR responses of young dogs with a history of normal or ischemic uterine environments. Uterine ischemia was produced throughout gestation by mechanical restriction of the uterine arteries and ligation of major collateral vessels. Beginning at 5 to 6 weeks of age, the dogs received 3 days of adaptation to the test environment, 2 days of CS-alone training, 4 days of discrimination conditioning, and 3 days of reversal discrimination conditioning. Control dogs received a fourth day of reversal conditioning following an injection of either 1.5 g/kg of 30% v/v ethanol or an equivalent volume of saline. The CSs were 700 and 1500 Hz tones and the US a shock to the hind limbs. During each day of discrimination and reversal conditioning, the dogs received 16 trials with one of the CSs being paired with the US and 16 trials with the CS presented alone.

The main findings were that the HR responses of the experimental group were decelerative to the two CSs during the CS-alone, original discrimination, and reversal discrimination phases of the study. Positive evidence of conditioning in the experimental group was not obtained. The control group showed a triphasic HR response to both CSs during original discrimination conditioning that appeared to extinguish on the reversal conditioning trials. The response consisted of a brief period of HR deceleration, followed by HR acceleration, followed in turn by HR deceleration. The control group gave a slight indication of differential responding to the two CSs during original discrimination conditioning. During reversal conditioning, HR responding in the control group diminished to the CSs. Ethanol tended to reinstate HR responding. The experimental group typically demonstrated a monophasic deceleration to the shock US while the control group responded with a biphasic pattern, consisting of HR acceleration followed by HR deceleration. A final difference between the two groups concerned baselevel HR. Throughout the study, the control group had a higher baselevel HR than did the experimental group.

The pattern of experimental outcomes suggested that cardiac regulatory processes may not have developed normally in the experimental dogs having a history of intrauterine ischemia. It was hypothesized that intrauterine ischemia delayed the development of normal sympathetic control of cardiac activity.

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APPENDIX A

Hodgkinson, Hodari, and Bumpas (1967) reported that of the 45 dogs whose uterine arteries were banded, 31 became pregnant. Of the pregnant mothers, 20 were delivered by Caesarean section around day 58 of gestation (normal gestation is about 63 days) while 11 underwent abortion or premature delivery prior to this age. The last observation is of some concern because, as they note, abortions are thought to be infrequent in dogs. They also noted that fetal malformation was frequently observed although they failed to describe the litter size and rate of survival in the pups delivered by Caesarean section. Nevertheless results like these suggest that when one is doing research on prenatal effects and behavior, factors other than the ones thought to be directly manipulated may influence the experimental outcome (Joffee, 1969). Two such factors of concern in the present experiment are litter size and viability of offspring.

In the present experiment all births were natural (vaginal). It is interesting to note that impregnation of banded mothers appeared to be more difficult (6 of 12 dogs conceived) than in the non-operated control mothers (4 of 5 dogs conceived). This difficulty is probably not due to paternal factors, because semen from the same stud male was used for all pregnancies. The question of neonatal viability is more difficult to address in the present experiment because of the small number of pregnancies. In the 4 non-operated controls that conceived, there were litters of 7, 9, 9, and 5 puppies with 7, 2, 6, and 5 survivors in each of the litters, respectively. Only the infants from the last two groups were run in the present experiment. In the banded mothers that conceived, litter sizes at birth were 9, 8, 8, 5, 2, and one litter size unknown (≥ 3?), while in each of these litters 1, 0, 1, 0, 2, and 3 puppies survived, respectively. Again, only the puppies in the last two litters were run in the present experiment. The single pup in the first litter was used to determine the protocol for the present experiment. Based on this small data base, it is very difficult to conclude firmly that the two groups differed in litter size. A Mann-Whitney U test ($\underline{U} = 1.5$, $\underline{p} < .02$) does suggest however that viability was different in the two groups. The exact relationship of this finding to the experimental banding procedure is unclear, however. Nevertheless, these factors should be considered when interpreting the overall results of this experiment.

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