The Development of Classically Conditioned Heart Rate Responses to CSs Evoking Distinctive Orienting Responses

by

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Title: The Development of Classically Conditioned Heart Rate Responses to CSs Evoking Distinctive Orienting Responses.

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Two experiments were conducted that observed the effects of different CS modalities (light vs. tone) on both unconditioned and classically conditioned heart rate responses in an attempt to delineate the relationship between these two types of responses. In both experiments, heart rate was monitored in unrestrained rats before, during and after a classical Pavlovian conditioning procedure.

In the first experiment, an attempt was made to manipulate the spontaneous response to the light and tone by presenting one or two stimulus modalities during stimulus pre-exposure sessions. The CSs were then paired or explicitly unpaired with a shock sequence delivered subcutaneously in an attempt to establish classically conditioned responses. Non-reinforced CSs were presented after conditioning sessions so as to measure extinction effects.

Stimulus-specific heart rate responses were observed in unrestrained rats to the tone and light stimuli that did not habituate after 100 trials and were not affected by the number of stimulus modalities per session. Both responses to the light

and the tone began with an initial deceleration which changed to an acceleration. Responding to the tone returned to baseline levels before the end of the 28-sec stimulus but responding to the light remained accelerated throughout the CS. These responses were not affected by differential reinforcement with the subcutaneous US, therefore it was concluded that classical conditioning did not occur.

The second experiment attempted to isolate the reason for failure of conditioning in Experiment 1. A different location (the skin) was designated for the site of US delivery, known to contain more pain receptors. This shock was paired and unpaired with the light and tone CSs of Experiment 1 in a typical discrimination paradigm. Discriminative CRs developed within the first four conditioning trials that were were deceleratory to CS+ and acceleratory to CS-. These conditioned responses did not exhibit extinction after 20 non-reinforced trials per stimulus presented after conditioning.

It is of further interest to determine the proportion of response components determined by the US and what portion (if any) by the CS in future experimentation. It is proposed that by manipulating both the spontaneous response and the unconditioned response such a determination could be made.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Over the past 50 or so years, there have been many speculations in the scientific literature about the origin and development of the classically conditioned response. These speculations can generally be classified in one of four ways: (1) The characteristics of the conditioned response (CR) develop such that they resemble the characteristics of the unconditioned response (UR); (2) The characteristics of the CR develop so as to prepare or compensate for the upcoming UR ; (3) The characteristics of the CR develop out of the original or orienting response (OR) to the signalling stimulus or conditioned stimulus (CS); and, (4) The characteristics of the CR are a composite of attributes similar to both the OR and UR. In offering these suggestions, learning theorists have often presumed (perhaps incorrectly) that the observed similarity between CR and UR or CR and OR is indicative of an underlying causal relationship.

This thesis reviews the various methods employed to determine the relation between the CR and unlearned responses to the CS and US. In the experiments reported here, changes in the cardiovascular response system, specifically heart rate changes were observed. Heart rate ORs, CRs and URs were examined in a Pavlovian conditioning paradigm. The heart rate changes to stimuli of different modalities were analyzed for distinctive characteristics. These stimuli were either paired or explicitly unpaired with a shock stimulus and the effects of these pairings on responding were observed. Attempts were made to manipulate the topographies of the ORs to the different stimuli and the effects of these manipulations on the CRs were analyzed.

Of the theories that suggest the characteristics of the CR ought to resemble those of the UR, Pavlov's (1927) findings and the stimulus substitution theory derived from those and similar data are the standard model (as outlined by Mackintosh, 1974). Pavlov based his theory of CR development primarily on his observations in dogs. He found that classically conditioned salivary responses resembled the unconditioned salivatory response, i.e., both were increases in salivation from previously measured baseline levels. He proposed that the occurrence of a CR resembling the UR was due to increased association between the two cortical areas stimulated by the CS and the US such that the CS was now capable of eliciting a response normally evoked by the US.

Pavlov's results (URs and CRs that resembled each other) were replicated by Schlosberg (1936) based on foreleg shock in the rat. However, extension of the classical conditioning paradigm to other response systems (including the cardiovascular system) questioned whether an increase in responding to the US was always accompanied by a similar kind of increase in responding to the CS. For example, Subkov and

Zilov (1937) found an acceleratory heart rate UR but a deceleratory CR. Such bidirectional responding was not found in the above examples of salivary and motor conditioned responses (e.g., a decrease in salivation or leg flexion below baseline levels). Closer examination of the response systems involved suggests a reason for this discrepancy. Levels of baseline responding for salivation and leg flexion are characteristically low in untrained subjects. Thus, one might not easily detect reductions in salivation or leg flexion simply by measuring differences from untrained levels of baseline responding. Given that heart rate and blood pressure baselines are usually much higher than zero in the normal animal, it is much easier to detect response changes below baseline (Subkov & Zilov, 1937). Although conditioned responses of the cardiovascular system were believed by many to be artifact (Shearn, 1961), these findings prompted numerous investigations of heart rate conditioning and led to other theories of CR development.

Schneiderman (1974), a proponent of the second position, viewed the CR as an anticipatory compensatory response to the UR and supported his theory with many examples of CRs and URs of opposite direction. He conceptualized the CR as an adaptive response that prepared the organism to either augment or compensate for the effects of the US, depending on the constellation of URs it elicited. Thus if the UR was a large increase in heart rate, the CR would be a decrease so as to alleviate the consequences of the large acceleration. However, in the cardiovascular system, the direction of heart rate CRs is jointly influenced by blood pressure and somatomotor characteristics of the UR. Heart rate CRs and URs of similar direction were explained according to Schneiderman as augmentation of blood pressure or somatomotor movements by the CR instead of direct compensation for the heart rate UR. For example, if the US in a particular experimental situation led to prolonged movement with a concommitant demand for increased energy expenditures, then the cardiovascular CRs would augment the cardiovascular URs (e.g., both would be accelerations) rather than compensate for the acceleratory UR.

The third major viewpoint mentioned at the start of this paper focuses on the unconditioned response to the novel stimulus, a response termed the orienting response (OR), as a determinant of characteristics of the conditioned response. A detailed consideration of heart rate ORs to various stimuli is included later in this paper, but the main thrust of this theoretical position is that the CR is an enhanced OR that develops through a sensitization process which is activated by pairings with the US (Dykman, 1967; Hoffman & Fitzgerald, 1978; Sokolov, 1963). Accordingly, the topography of the CR would be expected to be similar to that of the OR.

The fourth theoretical possibility mentioned above is that CR development is not influenced by one, and only one factor of the whole conditioning procedure but rather is influenced by many components of the experimental procedure. The foremost of these influences would be the reactions to the novel stimulus and to the unconditioned stimulus. Two advocates of a composite CR are Holland (1980) and Wilson (1969), although Dykman (1967) also mentions the possibility of other response components gradually adding to the CR. These other components were thought to be determined by parameters of the conditioning procedure other than the CS but were not explicitly stated as being due to the US or UR.

Holland (1980) classically conditioned rats using lights and tones of various duration as the CSs and delivery of a food pellet as the appetitive US. He found that responses resembling parts of the UR (e.g., placing the snout in the food magazine) occurred toward the end of the interstimulus interval, while behaviors evoked differentially by auditory and visual stimuli prior to conditioning (e.g., startle behavior to a tone and rearing behavior to a light) occurred mainly near CS onset during conditioning. He proposed that the CS-US contingency results in the acquisition of two separately elicited classes of behavior, one determined by and appropriate to the US, and the other an enhancement of the OR, with the overall pattern of behavior depending on the interaction between these two classes of behavior. In other words, an interaction between CS and US determined behaviors may result in a new combination of behaviors to the CS (e.g., a significant amount of head-jerk behavior to the tone in addition to startle and magazine-oriented behaviors). Holland did not consider autonomic responses such as heart rate, but it is of interest whether such responses would follow the same developmental pattern as do overt responses.

Wilson (1969) proposed a multi-component theory of CR development similar to Holland's in a study which recorded pulse-rate in man during stimulus pre-exposure and classical conditioning. He found a distinctive inverted U-shaped response to a tone CS, and after subsequent tone-shock pairings, a CR that resembled the original response to the tone in direction and topography but was significantly enhanced. Wilson believed the enhancement was due to the occurrence of the US. This study will be described in more detail below, but in brief, he stated that the interaction of the initial "spontaneous response" to the CS and the unconditioned response to the shock determine the conditioned heart rate response (with the former being the more influential). With training, the composite response curve resulting from these two inputs has added to it a further component that is specifically linked to the signalling function acquired by the CS. After repeated pairings, the CS initiates a preparatory set (an expectancy of the stimulus to follow) that focuses on the occurrence of the US at a specific point in time. When administered, the US terminates the trial exactly as expected, to which Wilson attributes the enhancement of the spontaneous response.

This is very similar to a cognitive interpretation of learning (e.g., Grings, 1960) except that Wilson superimposes the US upon a "spontaneous response" to the CS rather than on baseline responding as is usually implied. It is important to note that Wilson assumes a causal relationship based on the correlation between CR and OR topographies. This issue will be discussed later.

A possibility that Wilson did not mention was that an explicitly unpaired CS could also initiate a preparatory set in the same manner as a CS consistently paired with the US, but instead with an expectancy for the US not to follow. In this case, the nonoccurrence of the US at the end of an explicitly unpaired stimulus trial (CS-) would also terminate the trial exactly as expected, reinforcing the preparatory set. If as proposed by Rescorla (1969), the CS- response is opposite to the CS+ response, one might expect an attenuation of the spontaneous response to the CS- and for this attenuation to increase with increasing numbers of unpaired CS- trials.

The Orienting Response

Sokolov (1963) defined the OR as a reaction that: (a) is non-specific with regard to the quality of the stimulus, (b) exhibits adaptation with repeated presentations of the stimulus, and (c) is non-specific with regard to the intensity of the stimulus. This implies that the OR to many different types of stimuli should always be similar in direction and shape if measured in the same way. Therefore one would expect all responses to stimulus presentations measured before conditioning trials to be either heart-rate accelerations or decelerations independent of experimental design, but numerous studies have found conflicting results with various stimulus intensities and modalities.

Heart rate ORs to a tone CS were both accelerations and decelerations in 24-wk-old human infants (Lewis, Kagan, Campbell & Kalafat, 1966), decelerations to both lights and tones in 6-mo-olds (Lewis & Spaulding, 1967), and accelerations to tones in newborns but decelerations in 6-mo-olds (Berg, 1972; Clifton & Meyers, 1969).

Rather than discount Sokolov's theory, Berg (1972) hypothesized that all cardiac decelerations were orienting responses to the novel stimulus and that recorded accelerations were, in actuality, defense reactions by the subject. This view was supported by Graham and Clifton (1966) and Lacey and Lacey (1958) who suggested that the heart rate decelerations were associated with stimulus enhancement or attention and the accelerations with stimulus rejection. Also, the acceleratory defense reaction was thought to be non-specific to the quality of the eliciting stimulus but depended on stimulus intensity. In contrast, the deceleratory OR was thought to be uninfluenced by either stimulus quality or intensity. The defense reaction was evoked by "strong stimuli" and its function was to "limit" stimulus action, thus defending the organism from excessive stimulation. The virtually universal finding of heart rate acceleration to intense electric shock was offered as an example of a defensive reaction. Following the above line of reasoning, the function of a shock-induced acceleration is to decrease the excessive stimulation caused by the shock. However, less detailed evidence was available on the accelerative response to other intense stimulation (e.g., loud sounds) but it was assumed that the pattern of heart rate changes found with shock and noise was representative of a defense reaction characterized by prolonged acceleration.

Graham and Clifton (1966) therefore concluded that all studies showing an acceleration as the heart rate response to a stimulus must have used CSs of relatively high intensity that were eliciting defensive responses. Studies that reported biphasic responses (Geer, 1964) were explained in similar terms, e.g., as a shift from a deceleratory OR to an acceleratory defensive response due to a high intensity stimulus or "prepain zone of intensity". This was the interpretation of a study by Black (1964) who monitored heart rate in rats during a 40 dB white noise presentation. When the greatest difference between heart rate in a 3 sec pre-stimulus period and in any 3 sec of a 20 sec post-stimulus period was measured, a deceleration was found in the first few trials that changed to an acceleration in subsequent trials. It is unclear according to this interpretation why a stimulus benign enough (e.g., of 40 dB intensity) to initially elicit a decelerative response should cause a subsequent acceleratory defense reaction when no aspect of the stimulus was changed. Graham and Clifton (1966) explained this as a habituation of an initial deceleratory OR that then left unobscured an acceleratory defense reaction. It is of interest to note that the method of scoring heart rate changes used by Black could not detect a biphasic response occurring within a single CS presentation but could only indicate which direction of the response predominated in a given trial.

Wilson (1969), rather than accept a post-hoc analysis of orientng responses, sought a different explanation of bidirectional heart rate responding. He recorded a consistent inverted U-shaped heart rate acceleration in humans exposed to a discrete tone stimulus. Because this response did not easily habituate, contrary to Sokolov's definition of the OR as mentioned above, Wilson called it the "spontaneous response" to the stimulus.

In a large and thorough experimental design, he described the direction and form of the spontaneous response to various stimulus modalities and intensities, the rate of its habituation as compared to more transitory components of the orienting response repertory, and how it is affected by other stimulus events (e.g., pairings with an aversive shock or loud noise).

Wilson described a spontaneous cardiac response whose form, magnitude and phasing are affected by the properties of the stimulus and the structure of the experiment. Initially, the novelty and uncertainty associated with the beginning of the experiment produced a change in baseline heart rate different in form from the subsequently occurring spontaneous response. This initial change persisted for only a few trials and rapidly habituated in accordance with definitions of the OR. As the initial effects of novelty and uncertainty dissipated, heart rate responding to the discrete stimulus was gradually converted to the spontaneous response. After a sufficient number of trials, Wilson reported that the spontaneous response also "habituated," but retained its characteristic form throughout habituation, i.e., habituation was defined as a decrease in the absolute magnitude of the response rather than total abolition.

The more transient initial response that Wilson described as occurring on the first few trials of stimulus pre-exposure fit Sokolov's definition of an OR due strictly to novelty. The habituation of this response may then leave an initially masked acceleratory spontaneous response with a slower habituation rate. This is an alternative explanation to Graham and Clifton's (1966) of decelerations that turned into accelerations with repeated exposure to the CS. Rather than a change from an orienting response into a defensive reaction due to some ill-defined threshold of stimulus intensity, the transformation of deceleration into acceleration could be the habituation of an OR, thereby unmasking a spontaneous response.

Wilson described the attenuation of the initial OR as a function of a general decrement in responsiveness to the total experimental situation plus a more specific habituation to the stimulus itself. He then went on to emphasize the need for an extensive study of the longer-lasting spontaneous responses to unreinforced stimuli and argued that one of the most notable omissions from previous studies has been a complete analysis of the response to the CS alone in stimulus pre-exposure sessions.

There are only a few studies that have looked at heart rate responding to unreinforced auditory stimulation in rats (Black, 1964) and in humans (Chase & Graham, 1967). Both studies found an average deceleratory heart rate change in early trials that changed with stimulus repetition to an average acceleration. In a study using tones presented to restrained rats, Fitzgerald and Hoffman (1976) found that increased repetition led to a greater degree of habituation of the deceleratory component of the OR. The greater the habituation of this component, the greater the magnitude of the conditioned deceleratory response on subsequent reinforced trials. This indicates a possible detrimental effect of the deceleratory component of the OR on the developing CR despite the directional similarity of OR and CR in this preparation. Unlike Chase and Graham (1967) and Black (1964), Fitzgerald and Hoffman did not observe a significant acceleration at the end of habituation or at any other time, although the group receiving 50 CS alone trials showed slight accelerations by the start of conditioning trials.

Fitzgerald and Hoffman reported a mean heart rate change score averaged over the 6 sec ISI. This made it impossible to detect a biphasic response since an acceleration present during one part of the ISI would be obscured by a larger deceleratory component in another part of the ISI when the average response was calculated. If, as Wilson suggested, a characteristic response to the tone were present, it would become more obvious with repeated exposures (as the novelty of the situation habituated). The chances of the acceleration overcoming the decelerative component in an averaged response would be greater with increased number of stimulus exposures. The response when averaged, would then be an acceleration. The relatively short ISI in this study and the use of an average change score puts obvious limitations on the interpretations of these data.

It is also important to note that the restraint used by Fitzgerald and Hoffman may have had an enhancing effect on decelerative components of heart rate responses. This issue will be dealt with below in the discussions of heart rate responses to specific types of stimulation.

It appears from the literature review that a more thorough experimental evaluation of the characteristics of cardiac responses to novel stimuli is in order. In pilot work, the heart rate response to a 28-sec continuous tone CS was found to be of a different pattern than that to a 28-sec light CS which was presented intermittently (i.e., 2 flashes per sec). These responses were present both before and after classical conditioning with an electric shock US. Although our observations of heart rate responding to a continuous light and an intermittent tone were not extensive, it appeared that the response topographies were not changed by the manipulation of onset-offset pattern. Therefore, it seems as if the difference in responding to the light and tone is mostly attributable to modality rather than onset-offset pattern differences.

A review of the literature supported the notion that modality-specific heart rate responses exist, but did not reveal any evidence of characteristic cardiac responding to intermittent as opposed to continuous stimuli. Characteristic spontaneous responses to different CSs (whether due to modality or onset-offset pattern) would allow for a more definitive evaluation of possible similarities between original and conditioned cardiac responses.

The Heart Rate Response to a Light Stimulus

The heart rate responses to visual stimuli are not well studied. Of the investigators using visual CSs in classical heart rate conditioning studies (Cohen & MacDonald, 1971; deLeon, 1964; Porges, Stamps & Walters, 1974; Smith &

Stebbins, 1965; Tighe, Graves & Riley, 1968), only two included any detailed analysis of the shape or direction of the unconditioned response to a non-reinforced light (Cohen & MacDonald, 1971; Porges et al, 1974). This severely limits any conclusions that can be drawn concerning the characteristics of orienting responses, spontaneous responses and the persistence of these characteristics in conditioned responses that develop to a reinforced light.

One of the first studies that measured heart rate changes to a light in a classical conditioning paradigm was by deLeon (1964) using human subjects. He paired a 1-sec light CS with a 6-sec high intensity noise US in an attempt to replicate the heart rate conditioning findings of Notterman, Schoenfield and Bersch (1952) who had used a tone CS and a shock US. After 11 pairings (ISI = 6 sec), the light began to elicit a decelerative heart rate response (averaged over the entire CS-US interval). The characteristics of the CR in extinction, spontaneous recovery and reconditioning paralleled the findings of Notterman, et al. Both findings corresponded with Pavlov's descriptions of CRs during such behavioral tests and therefore, deLeon concluded that this deceleration was a response due solely to CS-US pairings rather than to psuedoconditioning or sensitization of responding.

The OR to the light in deLeon's study was also a deceleration when averaged over the 6 sec following the light. It should be noted that the light CS was not presented throughout this entire measurement interval but only during the first second. The magnitude of this response diminished with repetition and was essentially gone by the end of the 20 stimulus pre-exposure trials. DeLeon did not look at the topography of the response over the ISI, making it impossible to observe any multiphasic components.

Tighe, Graves and Riley (1968) classically conditioned heart rate using two intensities of light, one of which was paired and the other explicitly unpaired with shock, in two restrained rats. The conditioned response was a consistent deceleration of at least 40 bpm measured as the difference between heart rate at the start and at the end of an 11 sec ISI. Rats were given over 2000 conditioning trials, during which 18 reversals of CS+ and CS- took place. When a stimulus was changed from a CS+ to a CS- the deceleratory response was significantly diminished and when a stimulus that had served as a CS- was subsequently reinforced, the CR to that stimulus became a substantial deceleration.

The original responses to the two lights were measured during 30 non-reinforced trials each. Although no detailed data were furnished on these responses, accelerations occurred to both lights. The magnitude of these accelerations were not described other than a mention that the 20 bpm acceleration on the thirtieth presentation was diminished as compared to accelerations on earlier trials. Responses to the shock were accelerations of 90 bpm.

Cohen and MacDonald (1971) found a prolonged acceleration in heart rate during 10 presentations of a 6-sec light in restrained pigeons. This was measured for each 2 sec interval of the CS. When 40 presentations were given, a short latency heart rate increase was found that habituated rapidly, although the longer latency accelerative component was still present at the end of 40 trials. Intermittent footshock modified this response when unpaired with the light by producing a decelerative component that persisted for only a few trials. Pairings of shock and light resulted in accelerative CRs that were not very different from pre-conditioning responses to the light (i.e., there was no mention of transient deceleratory components of responding). The accelerative heart rate response therefore remained thoughout testing and its amplitude over the CS presentation was a monotonically increasing function of time such that the maximum acceleration was at the end of the CS.

Smith and Stebbins (1965) also found an increase in heart rate to a light paired with shock in restrained monkeys. The light in this case, was 56 sec long and the shock occurred simultaneously with light offset. The conditioned cardiac response to the visual CS+ was an acceleration of relatively long duration (30-60 sec), that increased in magnitude as the CS progressed. After reaching a maximum (at least 30 sec after CS onset), the response diminished, tending to return to baseline prior to the end of the 56-sec ISI. Acceleration was not seen to another light explicitly unpaired with the shock. Instead, heart rate did not differ significantly from baseline during the CS-, suggesting a possible attenuation of the original response to that light. Unfortunately, no data were included that described CS-alone responding to either light, making it impossible to compare the CRs and ORs in this study.

Porges, Stamps and Walters (1974) measured response patterns to the onset and offset of a 30 sec increase in illumination in human newborns. Only subjects with a high pre-trial variability of baseline heart rate (which they proposed was an index of neonatal maturation) responded significantly to changes in stimulation. The response to light onset was characterized by a significant quartic trend containing both accelerative and decelerative components. A small decrease was seen for the first 10 sec of the stimulus, the response then changed direction to become an acceleration of greater magnitude than the previous deceleration. The acceleration was at its maximum when the sampling period was terminated (30 sec after CS onset). The effects of pairing the CS with an aversive US were not studied.

It is difficult to draw conclusions about the diverse responses recorded to light stimuli due to the small number of studies concerning them in the literature. Out of five studies in which heart rate responses to light stimulation were measured, all but one included descriptions of ORs to the light before pairing with the US. The responses recorded varied from a deceleration in adult humans, to a biphasic response in newborn humans, to accelerations in rats and pigeons. Powell, Schneiderman, Elster and Jacobson (1971) measured both acceleratory and deceleratory CRs to visual CSs that were independent of reinforcement schedule, but did not describe ORs to these stimuli in any detail. Of the four studies in which responses to lights after pairings with USs were measured, decelerations were found in humans and rats, but accelerations in pigeons and monkeys. No conclusive evidence is thus furnished to support any consistent correlation between ORs and CRs since the above studies found ORs and CRs of both similar and opposite direction.

A point that may help clarify the cause for such differences is the time course over which the CS is measured and how the response is scored. deLeon (1964) measured HR over the shortest period (6 sec) and found deceleratory ORs and CRs. Tighe et al. (1968) measured over a slightly longer period (11 sec) and observed an acceleratory OR and a deceleratory CR. Smith and Stebbins (1965) and Porges et al. (1974) used much longer periods (30-56 sec) and observed accelerations. Porges et al. (1974) showed that an initial deceleration occurred in the first 10 sec of the response that changed with continuation of the ISI into an acceleration. It is possible that short ISIs limit the possible responses observable (i.e., multiphasic responses). Tighe et al. (1968) also used restrained animals. This may affect directionality of responding and will be discussed later.

When an acceleration occurred as the OR or the CR, it appeared to increase for the first 20-30 sec of the CS and when recorded for longer periods, heart rate returned to baseline after 30-60 sec. Acceleratory ORs did not habituate as rapidly as did the deceleratory ORs but persisted after 30 trials as measured by Porges et al. (1974) and in Cohen and MacDonald (1971). Deceleratory ORs were reduced to baseline levels of responding within 20 trials.

The diversity of these findings may also be due to species, the amount of pre-conditioning trials and other factors not clearly described in the methods sections (such as restraint condition). Clearly, there is a need for more intensive studies of both the OR (and the spontaneous responses) and the conditioned heart rate responses to visual stimulation.

The Heart Rate Response to a Tone Stimulus

The literature concerning heart rate responses to auditory stimulation (either reinforced or not) is of a greater volume than that concerning visual stimulation, and a wide diversity of response patterns is evident. An increase in average heart rate with respect to pre-CS baseline rates during conditioning trials was found in restrained dogs (Dykman &, Gantt, 1959; Fitzgerald, Vardaris & Teyler, 1966; Fitzgerald & Walloch, 1966; Sutterer & Obrist, 1972), restrained monkeys (Randall, Hasson & Brady, 1979), rats

(Martin & Fitzgerald, 1980), and in humans, (Cohen & Johnson, 1971; Wilson, 1969). Other studies have measured decreases in heart rate to tones in freely moving cats (Howard, Obrist, Gaebelien & Galosy, 1974), humans (Notterman, Schoenfield & Bersch, 1959 a and b), rabbits (Lockhart & Steinbrecher, 1970), and in both restrained and unrestrained rats (Fitzgerald & Martin, 1971; Fitzgerald & Teyler, 1970; Martin & Fitzgerald, 1980; Fitzgerald, Vardaris & Brown, 1966; Teyler, 1971). Powell, Schneiderman, Elster and Jacobson (1971) found both accelerations and decelerations to tone CSs in restrained rabbits. These discrepancies may be due, in part, to particular parameters of the experimental design, such as restraint condition, shock intensity and interstimulus interval length.

Teyler (1971) conditioned heart rate in rats using a paradigm that factorially combined two degrees of restraint condition with two locations of shock and three shock intensities. He found that for all subjects exhibiting learning, the CR was a deceleration with the magnitude of the deceleration greater in restrained rather than in unrestrained rats, and greater in rats receiving chest shock rather than foot shock USs. The ORs to the tone were 40 bpm decelerations that returned over trials to baseline levels. This deceleration was greater in restrained than in unrestrained animals.

The intensity of the shock had no effect on magnitude or direction of the CR. However, in other studies (Dykman & Gantt, 1959; Fitzgerald & Teyler, 1970), shock intensity was positively correlated with the magnitude of the deceleratory CR. Teyler (1971) did not record response topography over the 6 sec ISI, leaving unanswered the question of whether the restrained, chest shock animals exhibited a decrease in accelerative components or an increase in the magnitude or duration of deceleratory components of the response over trials. Both of these changes would result in decelerations of greater magnitude when averaged over the ISI.

Fitzgerald and Teyler (1970) analyzed the topography of the CR by computing average changes for each 2-sec interval of the 6-sec ISI and found that the magnitude of the heart rate decelerations generally increased with time until US onset. The OR was a 50 bpm deceleration that habituated by the start of conditioning trials. Teyler's (1970) data suggest that Fitzgerald and Teyler's paradigm (restraint plus chest shock) should have enhanced decelerative CRs during the 6-sec CS (the effect of restraint on the OR was not discussed). Neither of these studies addressed the quetion of what other components (if any) combine with the short latency deceleration to form the complete response to the reinforced tone. Evident in Fitzgerald and Teyler's study was a decrease in the rate of deceleration 3-4 sec after CS onset during both pre-conditioning and conditioning trials. This is consistent with a heart rate response that returns to baseline. Whether this return to baseline is indicative of the end of the CR (as they imply) or is actually a shift to a longer latency acceleratory response is an open question.

Martin and Fitzgerald (1980) showed a significant effect of restraint on the direction of the conditioned heart rate response in rats, but did not discuss the effects of restraint on original responses even though 12 CS-alone trials were given for each of the two tone stimuli. Unrestrained rats exhibited a monotonically increasing acceleration over the CS while restrained rats showed a deceleration to a CS+ that approached an asymptote near the end of the 6 sec ISI. This supports the suggestion that restraining a rat affects the response to a CS by prolonging or exaggerating heart rate decelerations occurring within the first 6 sec after tone onset. When rats are unrestrained, decelerations do not occur and therefore acceleratory responses are not obscured. Again, longer ISIs would be useful in observing longer-latency accelerations.

Other studies showing decelerations to a reinforced tone utilized ISIs of short duration (1-10 sec) and in most instances averaged over this interval (Fitzgerald, Vardaris & Brown, 1966; Howard, Obrist, Gaebelein & Galosy, 1974; Lockhart & Steinbrecher, 1970; Notterman,Schoenfield & Bersch, 1959 a and b). Fitzgerald et al.(1966) used restrained subjects while Lockhart and Steinbrecher (1970) did not specify the restraint condition of their rabbits. The other studies all obtained decelerative CRs to a tone in unrestrained subjects.

It is interesting to note that the longest ISI (10 sec) used in the above studies resulted in an average conditioned response that did not differ significantly from baseline (Lockhart & Steinbrecher, 1970). These authors concluded that no significant heart rate response occurred. However the possibility of acceleratory and deceleratory components that were of equal magnitude and cancelled each other out, might be considered.

Studies finding an acceleration as a heart rate CR to a reinforced tone all contained ISIs of 6 sec or longer. Fitzgerald and Walloch (1966) and Fitzgerald, Vardaris and Teyler (1966) both used a 10 sec CS-US interval to condition restrained dogs with a tone paired with shock and found an increasing acceleration over days. In both studies, at least 80 CS-alone trials were given prior to training. Unfortunately, the responses to these presentations were not described. The heart rate score was averaged over the ISI obscuring possible deceleratory components.

Studies using longer ISIs and including analyses of response topography have found an inverted U-shaped acceleration to a reinforced tone. For example, Sutterer and Obrist (1972) using a 15-sec ISI when conditioning restrained dogs found a response that in the first few seconds after CS onset did not differ significantly from baseline but became a significant increase in heart rate during the next few seconds of the trial. Towards the end of the CS-US interval, the rate of acceleration decreased along with the magnitude of the response until the response again resembled baseline heart rate. Responses to a CS- did not differ significantly from baseline. Although CS-alone trials were given, the response to them was not described.

Dykman and Gantt (1959) used a 10-sec ISI in restrained dogs and noted a response similar in shape to Sutterer and Obrist's. This response topography was evident even within the first few trials of conditioning, and became more pronounced as training progressed. The magnitude of the maximum acceleration varied directly with US intensity and CS intensity. These effects could not be interpreted clearly, since these two variables were confounded in this particular design. The ORs to the tones were accelerations, the average of which was calculated over 30 stimulus pre-exposure trials.

Randall, Hasson and Brady (1979) used a 60-sec tone as a signal for unavoidable shock in restrained monkeys and found the maximum acceleration (about 30 bpm) occurring before 30 sec of the CS elapsed. The acceleration then diminished but only in a few subjects did it return completely to baseline levels of responding. The CS was a 3.4 kHz tone of relatively high intensity that was presented to the subjects about 30 times or until the OR had habituated. The shape of this OR was not described. It may be that an increase in tone intensity increases the magnitude of the maximum acceleration of the CR (as implied by Dykman & Gantt, 1959). The effect of CS intensity on conditioned heart rate responding has not been conclusively determined.

Few studies have observed, in detail, heart rate responses to tone-alone trials. A study by Fitzgerald and Hoffman (1976) found that increasing the number of stimulus pre-exposure trials before conditioning trials, decreased the magnitude of the deceleration measured during the 6 sec ISI of subsequent reinforced trials. In the first conditioning trial, animals that received 50 CS-alone trials actually responded with a small acceleration that was not statistically significant.

The first few seconds of the CS elicited decelerations in studies by Fitzgerald and Teyler (1970), Fitzgerald, Vardaris and Brown (1966), and Teyler (1971) but produced baseline levels in studies by Dykman and Gantt (1959), Fitzgerald, Vardaris and Teyler (1966), Fitzgerald and Walloch (1966) and Sutterer and Obrist (1972). A distinguishing detail between these two groups of studies is the amount of stimulus pre-exposure given to the subjects. The number of CS-alone trials in the former studies never exceeded 20, while in the latter studies, at least 30, and on the average, 60, stimulus pre-exposure trials were given prior to the start of conditioning.

The effect of stimulus pre-exposure on subsequently conditioned heart rate responses is not clear. A review of the deleterious effects of CS pre-exposure on subsequent CRs, an effect termed latent inhibition (Lubow, 1973), did not include mention of autonomic responses. Since then, Fitzgerald and Hoffman (1976) systematically showed that an increased number of CS pre-exposures resulted in smaller magnitudes of deceleratory CRs in subsequent conditioning trials. This supports the general trend found in the reviewed studies that fewer pre-exposures result in deceleratory CRs and more exposures result in baseline level responding during the first portion of the CS. Wilson (1969) however, did not find evidence of any deceleratory response components in the inverted U-shaped accelerations to tones measured in humans after only nine stimulus pre-exposure trials (fewer than any of the other studies reviewed here). A complicating aspect to this study was the communication that could exist between experimenter and subject, allowing the latter to be forewarned of stimulus occurrence, possibly affecting the response to it.

Summarizing the data on heart rate responses to tone stimuli makes apparent another classification into two groups: (1) Heart rate decelerations occurring during ISIs of less that 6 sec; and, (2) heart rate accelerations occurring during ISIs of more than 6 sec. This effect was also apparent in the review of responses to lights. A biphasic response that consisted of a deceleration during the first few seconds of the CS followed by an acceleration during the last portion of the CS might be described as either an acceleration or a deceleration depending on the time period over which the response was measured and how responding was averaged over that period. However, evidence such a biphasic response is tenuous at best. A more detailed analysis of the development of multiple component heart rate responses over longer ISIs during stimulus pre-exposure sessions and conditioning trials is clearly in order.

It should also be noted that the degree of restraint is not an innocuous condition when considering determinants of heart rate responding to lights and tones but its effect is not consistent. Teyler (1971) and Martin and Fitzgerald (1980) showed that restraint enhanced deceleratory components measured in the first 6 sec of a tone CS. However, most of the above mentioned studies revealed acceleratory responses to tones using restrained subjects, and when analyses of the response topographies during the CS were available, no evidence of initial deceleratory components were seen. In response to light CSs, Smith and Stebbins (1965) recorded accelerative CRs in restrained monkeys, Cohen and MacDonald (1971) measured both accelerative ORs and CRs in restrained pigeons and Tighe, Graves and Riley (1968) found accelerative ORs but decelerative CRs in restrained rats.

Species variation may also be important when attempting to explain discrepancies of tone response topographies. All of the accelerations found in the above studies were measured in dogs, humans and monkeys, while the decelerations occurred in rabbits and cats. Rats exhibited both types of responses.

Summary

Although the above findings point to possible characteristics of distinctive responses to lights and to tones, no definite conclusion can be drawn from the literature. It is clear however, that a design that adequately addresses this task should include methods of data collection that do not exclude observations of multiphasic responses.

When measuring heart rate changes during a relatively long CS-US interval, it appears that the response to a light is often a sustained acceleration with maximum magnitude occurring between 30-60 sec after CS onset. This response topography has been reported for both reinforced and non-reinforced light presentations. The response to a tone often resembles an inverted U-shaped function when measured over ISIs longer than 10 sec. The maximum acceleration occurs soon after CS onset and responding then returns to baseline about 15-60 sec after CS-onset. This response topography has also been observed in both CRs and ORs. In most studies using ISIs of relatively short duration (6-10 sec), ORs and CRs to both lights and tones are decelerations of significant magnitude. The exception to this rule are the findings of Powell, Schneiderman, Elster and Jacobson (1971), in which acceleratory and deceleratory CRs were found to occur to both auditory and visual stimuli independent of reinforcement schedules. The responses were measured over the same time interval. One possible explanation of the differences in response direction may be the number of stimulus modalities presented during each session. These findings will be discussed in more detail below.

From the literature alone, it is unclear whether initial decelerative components occur in the first part of responses to either a light or a tone. In studies using ISIs of less than 10 sec, significant decelerations were consistently found. A topographical analysis of responding during ISIs of longer durations seldom showed evidence of this component. This discrepancy may be due to the amount of stimulus pre-exposure the animal received, but the effects of this variable on heart rate responding have not been described consistently in the literature.

The effects of restraint conditions are also inconsistent across studies. Direct comparisons of various degrees of restraint and their effects on heart rate conditioning imply that restraint causes or enhances deceleration to both lights and tones in early portions of CS-US interval. Restraint however, cannot be the sole determinant of deceleratory heart rate responses given the many findings of decelerations in freely moving subjects during conditioning (Howard, Obrist, Gaebelein & Galosy, 1974; Notterman, Schoenfield & Bersch, 1959 a and b) and accelerations in restrained subjects as described above.

CS and US intensity, species variation, and intertrial interval are among other factors that need to be considered as affecting the heart rate ORs and CRs. The effects of these factors must be analyzed in more depth before any conclusions concerning the formations of spontaneous and conditioned cardiac responses can be formed.

Rationale and Method Summary

The main issue of interest in this thesis is what contribution the spontaneous response makes to the form of the conditioned responses to the same stimuli. In pursuing this issue, one might ask the following questions: Is there an unconditioned, "spontaneous response" characteristic of an intermittent light presentation and, if so, at what rate does it habituate? Does a characteristic cardiovascular response exist to a continuous tone CS, and if so, what are its characteristics? Are components of these responses similar to components of responses measured after classical conditioning? How are these spontaneous responses affected by the US and/or the UR, and is a response of the same form elicited after the CS has been paired or unpaired with shock? And finally, can the characteristics of these spontaneous responses be manipulated in such a way so as to affect the characteristics of the CRs. In other words, if one changes the spontaneous response, does one also change the conditioned response?

There are few studies in the literature that show evidence of manipulating original responses to stimuli other than through habituation. Powell, Schneiderman, Elster and Jacobson (1971) classically conditioned heart rate responses to tones and lights with electric shock in restrained rabbits. They found that the two stimulus modalities could each cause an acceleration in some instances and a deceleration in others. These significant effects were not due to the stimuli serving as CS+ or as CS- but instead seemed attributable to an interaction between the CS-US contingencies and the number of stimulus modalities presented per training session. The specific heart rate responses observed by Powell et al. (1971) are summarized in Table 1. The direction of the heart rate response to a reinforced light was predominantly acceleratory when the CS- was of the same modality and deceleratory when the CS- was of a different modality. The response to the unreinforced tone was deceleratory when the CS+ was also a tone and acceleratory when it was a light. The direction of the responses to an unreinforced light and a paired tone were not affected by the modality of the other stimuli but the magnitude of these responses was affected. Thus the deceleration seen to a reinforced tone was greater when the CS- was also a tone rather than a light. The accelerations seen to the unreinforced light appeared greater when the CS+ was a tone as opposed to a light.

Powell et al. (1971) believed that the differences in direction of heart rate CRs were due to differences in the

Table 1. The direction and magnitude of heart rate responses to the light and tone CSs of the four groups observed by Powell et al. (1971). The number of arrows signifies the general magnitude of the response (e.g., a two arrow response is of a greater magnitude than a one arrow response).

Table 1. The general direction and magnitude of the heart rate responses measured by Powell et al. (1971).

Groups	*CS+*	*CS-*
L - L	L = ↑↑ HR	L = ↑ HR
L - T	L = ↓↓ HR	T = ↑ HR
T - T	T = ↓↓ HR	T = ↓ HR
T - L	T = ↓↓ HR	L = ↑ HR

number of trials before the CR developed in the different groups. Discriminatory heart rate CRs were observed during the first trial block of conditioning and were deceleratory in the T-T group. Discriminatory heart rate CRs were not observed until the second trial block in the L-L group and were therefore acceleratory. Similar reasoning was followed for the heart rate CRs of the T-L and L-T groups although the correlation between CR directions and latencies of the first discriminatory CRs was not as definitive in these latter two groups.

They did not consider the possibility that the difference in the directions and magnitudes of the various CRs was due to differences in responding to the stimuli that were present before conditioning trials. The original cardiac responses to the stimuli in pre-exposure trials was not recorded in detail to either the tone or the light, making it impossible to know if any differences existed before conditioning. If the presence or absence of a second stimulus modality during testing affected the characteristics of the spontaneous response to the first stimulus modality, then this may have affected the direction and magnitude of conditioned responding. It should be noted that this could not be the only determinant of the characteristics of the CRs however, since differences were seen in CR magnitude between the T-L and L-T groups, but no differences existed in the number of modalities presented to these groups during stimulus pre-exposure sessions. Pilot work performed in our laboratory supported the notion that the number of stimulus modalities presented during a test session could alter both ORs and CRs to auditory and visual stimuli.

In the first experiment reported here, heart rate responses to stimuli that were expected to evoke different spontaneous responses (a continuous tone and an intermittent light) were recorded. The responses to these stimuli were characterized during preconditioning exposure to the stimuli, during reinforced and unreinforced presentations of the stimuli (with electric shock), during extinction, and during exposure to one or two stimulus modalities during test sessions.

If the topographies of the conditioned responses resemble those of the spontaneous responses, this will support theories which suggest that the CR depends on characteristics of the CS. Moreover, if presentation of stimuli of two different modalities during pretest sessions affects the spontaneous response to the stimuli, and this effect remains apparent in CRs recorded during conditioning and extinction, then this would be strong evidence that the spontaneous response determines components of the conditioned response or that the shapes of both responses are determined in part by the same cause.

Specifically, unrestrained rats received classical heart rate conditioning using a tone and a light as the CS+ and CS-and a series of electric shocks as the US. Two preconditioning sessions were included in the design for the assessment of ORs or spontaneous responses to the light and the tone. In some groups, only one of the two stimuli was presented, either paired or unpaired with the shock during conditioning trials. In other groups, both stimuli were presented, on separate trials. One of the stimuli was paired with the shock and the other was not paired with it. Extinction sessions were also included after conditioning as a test of the resilience of the conditioned responses. Extinction trials also allowed for examination of responses after conditioning sessions without the potential confounding effects of the unconditioned response to the shock.

A relatively long ISI (20 sec) was used in the following experiments to provide a greater opportunity for examination of the different components of the heart rate responses and their relative latencies. However, whether an ISI of such a duration will be effective in establishing distinct classically conditioned responses is also of interest.

Method

Subjects

The subjects were 48 adult male albino rats, 70-110 days old, with a mean wt. of 428.4 g (SD = 45 g), purchased from Holtzman Distributors of Madison, Wisconsin. They were individually housed in a colony room with a normal 12 hr light-dark cycle and provided with food and water ad lib except during the 70 min training periods. These training sessions occurred during the light portion of the 24 hr cycle.

Surgical Preparation

Between 5-7 days prior to the start of testing, animals were administered atropine hydrosulfate (0.3 mg/kg, i.m.) and 10 min later were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). Two heart rate monitoring electrodes were then surgically implanted under antiseptic conditions. Each electrode lead consisted of an 8 mm diameter stainless steel star washer soldered to a 15 cm length of 26 AWG 7-stranded copper alloy hook-up wire with thermoplastic insulation (Beldon, 8505). The implantation was done by making two incisions through the skin, one dorsally, approximately 3 cm below and to the right of the base of the skull and the other ventrally, approximately 1 cm rostral to the left foreleg. All connective tissue around these incisions was carefully cleared with a sharp pair of scissors and the washers were then sutured with 000 silk onto the superficial muscle layers, positioned so as not to be directly underneath the incisions. A drop of Superglue adhesive was used in some of the surgeries to hold the lead wire onto the muscle in an attempt to decrease electrode movement.

Both electrode wires were run to and then through the dorsal incision, where they were looped once, close to the exit point and this loop was then sutured and glued to the superficial muscles. After application of about 5 mg Furacin (0.2 % nitrofurazone, a topical antibacterial agent in a water-soluble base), the incisions were closed, again using 000 silk suture.

The electrode wires were trimmed to 5 cm lengths from the exit point and then stripped of insulation for about 1 mm. They were then each soldered to 1 cm male connectors (Amphenol, 220-P02) and housed in a plastic plug (Amphenol, 221-1560). This plug was securely attached to an external saddle (Smith & Davis, 1975), leaving enough excess wire so as to avoid external tension developing from the animal pulling against the plug. The saddle consisted of two loops of 18 ga stranded insulated wire attached to a modified hose clamp. It fit around the animal's upper chest and back with the two loops placed around the forelimbs. Care was taken in fitting the saddles so that they did not rest on any incisions, cause extreme discomfort or shift.

Apparatus

Animals were tested in a 23 x 20.5 x 21 cm clear plastic container provided with wood shavings and ventilation holes. This container was placed in a larger sound-attenuating chamber (50 x 52 x 45 cm) where the stimulus presentations were made. A light was mounted on the wall of the chamber, approximately 38 cm from the center of the plastic container and a loud speaker was mounted next to the light at about the same distance from the center of the container.

Two lengths of 21 AWG wire with thermoplastic insulation (Beldon, 8504) were attached at one end to two female connectors (Amphenol, 220-S02) housed in an Amphenol plug. The other ends of the wire were attached to a swivel device (Cunningham, 1978) incorporated into the ceiling of the plastic container. The wires were protected by a stainless steel spring and firmly attached to the animal saddle with two alligator clips. Wires led from the swivel to a special-purpose quick-recovering amplifier (Mundl,1965) and then to a Schmitt trigger. This amplified the heart rate signal and converted it to a digital signal that was used by a PDP8/F computer to compute interbeat intervals (accurate to 20 msec).

The visual CS was a 28 sec intermittent (2 Hz, .25 sec on) white light (6 W, 120 VAC) that was normally off. The auditory CS was an 83 dB (re: 20 uN/m^2) 2 kHz continuous tone, as measured in the center of the plastic container by an Audio Spectrum Analyzer (IVIE Electronics Inc., IE-10A) on the C scale. The tone also lasted for 28 sec. The unconditioned stimulus (US) was a series of three .5 sec, 1.3 mA shocks (with an interval of 4 sec from shock onset to shock onset) provided by a Grason-Stadler shock generator (E1064GS). This shock generator consisted basically of a 350 VAC transformer in series with 268 kΩ and the rat. Thus if the resistance of the rat changed from 1 to 50 kΩ, the current actually varied from 1.3 to 1.1 mA. These shocks were delivered through the heart rate monitoring electrodes and a relay switched out the heart rate amplifier during shock.

Procedure

Rats were randomly assigned to six groups with eight rats each. Table 2 lists the eight groups and their experimental treatment during each phase of the experiment. A T or L in the group labels followed by a plus sign designates the modality of the stimulus paired with the US during conditioning trials (e.g., L+ or T+). A T or L followed by a minus sign represents a stimulus modality that was not paired with a shock or was explicitly unpaired with it (e.g., T- and L-).

All rats were subjected to two sessions of stimulus pre-exposure, six sessions of conditioning and two sessions of extinction, in that order, with successive sessions approximately 24 hr apart. Each of these sessions began with a 10 min adaptation period after which the stimulus presentations began.

The stimulus pre-exposure sessions (Column 2 of Table 2) each consisted of 10 trials of the light CS (Groups L+ and L-) or the tone CS (Groups T+ and T-) or 10 trials of each (Groups L+/T- and T+/L-).

Each conditioning session (Column 3 of Table 2) consisted of the same numbers and types of stimulus presentations as in each rat's stimulus pre-exposure session, however, the US was then consistently paired or unpaired with

Table 2. The experimental design and procedure of Experiment 1 for each of the six groups during stimulus pre-exposure, conditioning and extinction sessions. The symbol "L" signifies the light CS, "T" signifies the tone CS, "+" signifies the US presented either during a CS or alone, and "-" signifies the absence of the US during a CS presentation.

Table 2. Experimental Design and Procedure.

NOTE: 10 = number of presentations per session

(+) = three .5 sec 1.3 mA shock pulses

(−) = no shock pulses

	Group	Stimulus Pre-exposure (2 sessions)	Conditioning (6 sessions)	Extinction (2 sessions)
Single-Modality Groups	L+	10 L−	10 L+	10 L−
	L−	10 L−	10 L− 10 +	10 L−
	T+	10 T−	10 T+	10 T−
	T−	10 T−	10 T− 10 +	10 T−
Combined-Modality Groups	L+/T−	10 L− 10 T−	10 L+ 10 T−	10 L− 10 T−
	T+/L−	10 T− 10 L−	10 T+ 10 L−	10 T− 10 L−

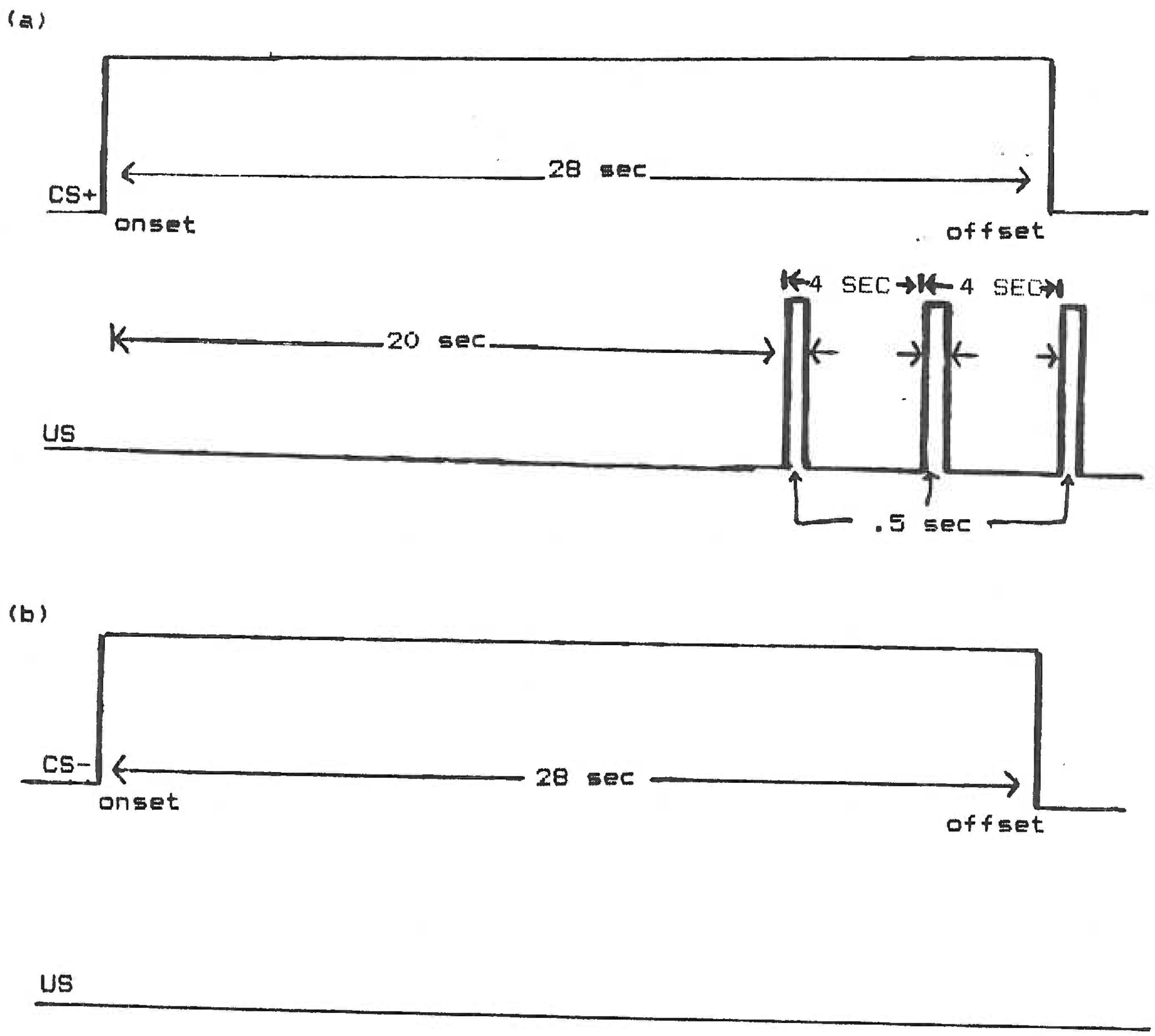
one or both of the CS events. Specifically, tone was paired with shock for Group T+ and explicitly unpaired with it for Group T-. The light was paired with the US for Group L+ and unpaired for Group L-. These four groups were designated the single modality groups. In the combined modality groups (L+/T- and T+/L-), a discrimination paradigm was used with the first stimulus in the group label paired with the US and the second stimulus presented without the US.

For the CS+ presentations, US onset occurred 20 sec after CS+ onset and consisted of three .5 sec pulses of shock separated by 3.5 sec intervals. Thus, the onset of the last shock pulse was simultaneous with CS+ offset (see Figure 1).

Extinction sessions (Column 4 of Table 2) consisted of 10 trials of the light alone or tone alone (Groups L+, L- and T+, T-, respectively) or 10 trials of each for the combined modality groups. No US presentations were made for any group during extinctions sessions.

The average intertrial interval for the single modality groups was 6 min and for the combined modality groups was 3 min with actual ranges of 90-540 and 90-270 sec, respectively. The mean interval between USs for all groups was 6 min. Appendix Table A1 presents a list of quasi-random ITI sequences that were used in order (1, 2, 3, 1,...) starting with List 1 for the first stimulus pre-exposure session. The different stimuli were presented in a semi-random sequence so that no more than three trials of any one type occurred

Figure 1. A schematic diagram of the temporal relationship between the CS and the US during (a) the CS+ trials and (b) the CS- trials.


(a)
CS+
28 sec
onset
offset
US
20 sec
4 SEC
4 SEC
.5 sec
(b)
CS-
28 sec
onset
offset
US

consecutively and so that each type of trial (e.g., L+) was preceded by the same type (e.g., L+) and by the other type (e.g., T−) equally often. Four lists were constructed to conform to these standards according to the method of Fellows (1967) and are presented in Appendix Table A2. These sequences were used in order (A, B, C, D, A,...) starting with List A for the first session. This ensured no repetition of any particular ITI-stimulus sequence combination during the 10 days of testing.

Six animals (i.e., one squad) were scheduled to be tested per day with one animal from each group represented. However, due to the elimination of 8 subjects after surgery or the start of testing due to death or bad electrode placement, this schedule was not maintained. In some squads, a group was represented by more than one subject, while in other squads a group may not have been represented at all. Appendix Table A3 lists the squads, their composition in terms of group representatives, and the approximate time of day each representative was tested (i.e., morning vs. afternoon).

Animals were ordered for three squads at a time except the last set which included enough animals for four squads. Therefore one third of the rats started testing 7 days, one third 17 days, and the other third 27 days after they emerged from quarentine after shipping. The last 7 animals were ordered at a younger age (35 days vs. 65 days) and did not start testing until 37 days after emergence from quarentine.

Data Analysis

A detailed analysis of successive recording periods was made possible by the method of on-line recording of each interbeat interval by the PDP8/F computer. These IBIs were translated into an average heart rate (BPM) for each 2 sec measurement interval during the trial.

Two criteria were used by the computer to distinguish signal from noise: (1) If the length of the IBI fell outside a range from 100 to 300 msec; and, (2) if the change in IBI duration from the previous IBI was so great as to question its authenticity. A limit was imposed upon the variability that could exist between subsequent IBI lengths. Interbeat intervals were classified by duration into latency bins, 20 msec wide (e.g., 100-119 msec, 120-139 msec, etc.). If an IBI was classified into a bin more than one adjacent interval in either direction from the classification bin of the previous IBI, then it fulfilled the second criterion.

Intervals that met either of the two above criteria were ignored, and a mean BPM score was derived from the remaining "good" IBIs of that 2 sec counting period. If the total duration of discarded intervals was greater than two thirds of the sampling period, then that whole 2 sec interval was discarded. For the statistical analysis, the following computed score was inserted in place of the discarded data. The mean change score was computed from the sum of the 2 sec intervals (corresponding to the discarded 2 sec interval) of the other subjects in the group of the animal whose data was discarded. The degrees of freedom were then properly adjusted according to the method of Linton and Gallo (1975).

A baseline heart rate was determined for each trial from the average interbeat interval during the 10 sec preceding the CS on that trial. Baseline heart rate was subtracted from the average heart rate for each 2 sec counting period, resulting in a set of difference scores for each trial. A positive difference score thus represents an acceleration from the baseline heart rate and a negative score a deceleration during that 2 sec interval.

Heart rate monitoring also occurred for six 2 sec counting intervals after CS offset and difference scores were also computed for these intervals. This allowed for an analysis of the response to the final shock and to CS offset.

Results

Separate analyses of variance were run on the mean heart rate change scores for stimulus pre-exposure sessions, conditioning sessions and extinction sessions. The first 14 2-sec counting periods of each trial during the pre-exposure sessions (i.e., the entire CS presentation) and the first 10 2-sec counting periods of each trial during the conditioning and extinction sessions (i.e., the CS-US interval) were designated as responses to the CS. The last 8 2-sec counting periods (not including those during which a shock occurred) of each trial during conditioning sessions were designated as responses to the US. Although the difference in measurement interval between stimulus pre-exposure sessions and conditioning and extinction sessions (28 vs 20 sec) precludes direct comparisons of responses during these sessions, general comparisons were still made. However, in none of the following analyses were differences seen when responses analyzed for 28 sec as compared to the same data analyzed over the first 20 sec. Therefore, the extra 8 sec was included for the additional information it revealed concerning the durations of the responses observed.

For the pre-exposure sessions, response patterns (measured over counting periods - CPs) to the light and the tone stimuli were compared between and within groups as were the response patterns between single vs. combined groups (S/C), and between groups designated as having the US paired or unpaired with the CS during subsequent conditioning trials (+/-). This last comparison assessed the differences between the paired groups and the unpaired controls that existed prior to conditioning due to random sampling error. It was also of interest to compare the changes in responding over five-trial blocks (TBs) of pre-exposure in order to make observations about rate of habituation of the original responses to the stimuli.

During condititioning sessions, the above comparisons were again made, although differences between responses to CS+s and CS-s (different from those seen in pre-exposure sessions) were examined as evidence of conditioning attributable to differences in CS-US contingencies. Changes in these responses across 10-trial blocks were examined for evidence of increases in magnitudes of changes attributable to increased number of reinforced or non-reinforced trials.

The same comparisons were again of interest in the analyses of responding during extinction sessions, however, the effect of trial blocks was examined for evidence of a decrement in any response changes seen to develop during conditioning trials. This would be consistent with classical notions of decrements in the magnitude or probability of CRs during extinction training.

To address these various issues, fourway analyses of variance (ANOVAs) were run separately on the data from the light presentations and from the tone presentations using number of CSs of different modalities (S/C) and reinforcement schedule (+/-) as the between group variables and consecutive trial blocks (TBs) and 2-sec counting periods (CPs) as the within group variables. Separate fourway ANOVAs were also run on the data from the single modality group stimulus presentations using modality type (L/T) and reinforcement schedule (+/-) as the between group variables and TBs and CPs as the within group variables. Finally, fourway ANOVAs were run on the data from the combined modality groups using reinforced modality (L+/T+) as the between group variable and reinforcement schedule (+/-) and TBs and CPs as the within group variables. All of the above analyses were run for each of the three experimental phases: stimulus pre-exposure, conditioning and extinction.

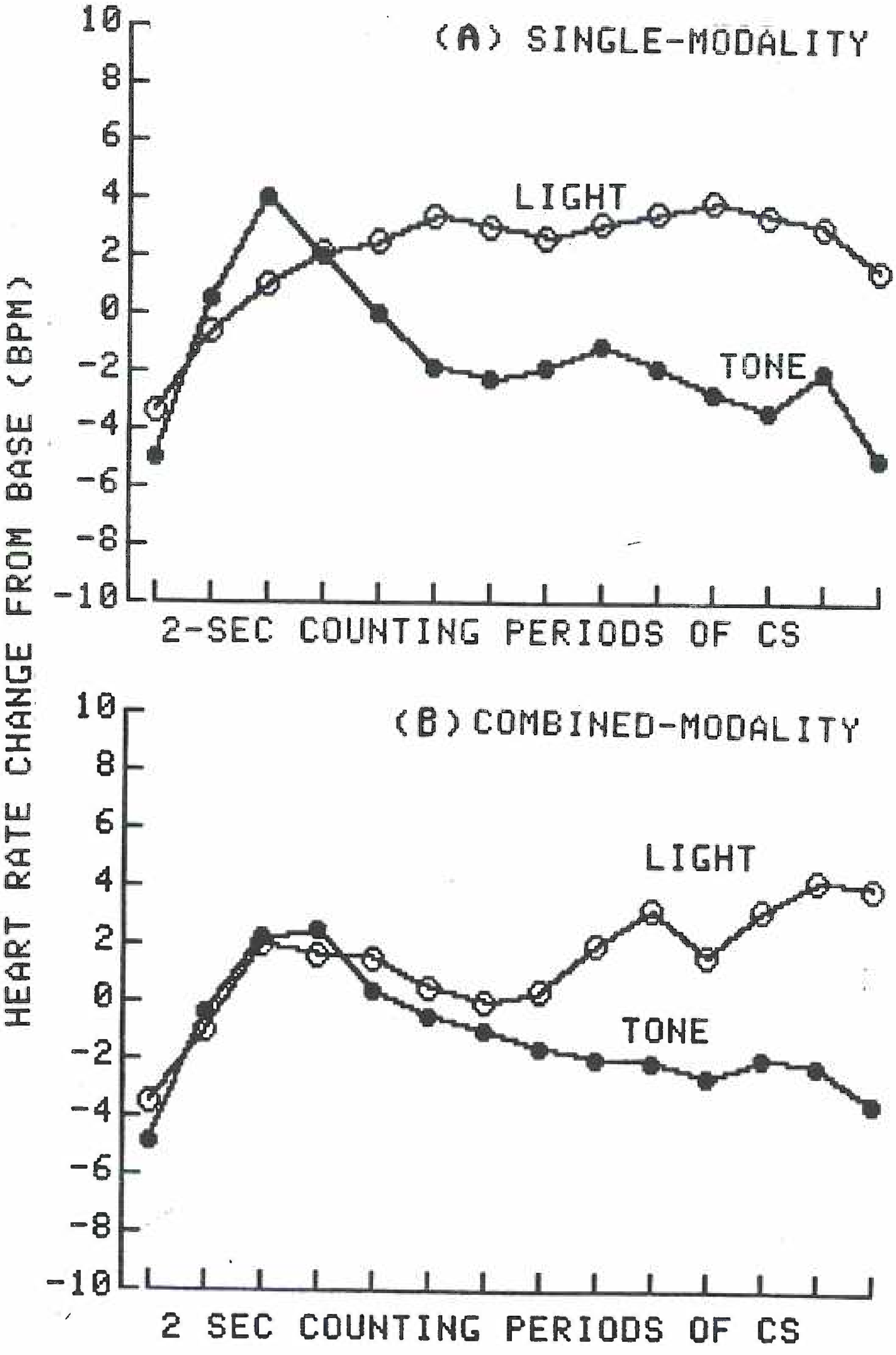
The strategy described above uses separate ANOVAs to assess light/tone differences and separate ANOVAs to assess single/combined differences. This strategy was chosen because it addressed two of the a priori comparisons in a direct manner and because it was not complicated by the fact that there were two within group variables in the single-modality groups and three in the combined-modality groups. An alternate method of dealing with this last problem would be to analyze the data in a large factorial analysis after subdividing each of the combined modality groups into two independent groups (e.g., L+, T-, etc.), ignoring the data from the "extra" CS. However, this would decrease the number of subjects in each of the "new" combined groups to half that of the other groups. The first strategy was therefore chosen because of its greater power (due to equal sample sizes in all groups) and because no data were discarded.

The α level was .05 in all analyses.

STIMULUS PRE-EXPOSURE: Light vs. Tone

Single Modality Groups. The mean responses to the light and the tone during adaptation for the single modality groups are shown in Figure 1a averaged over +/- (a dummy variable at this point) and TBs variables. Responses to both stimuli began with an initial deceleration of similar

Figure 1. The mean heart rate responses during stimulus pre-exposure sessions to the light and tone averaged over TBs and +/- variables. Single-modality groups are graphed in Panel (a) and combined-modality groups are graphed in Panel (b).


(A) SINGLE-MODALITY
LIGHT
TONE
2-SEC COUNTING PERIODS OF CS
(B) COMBINED-MODALITY
LIGHT
TONE
2 SEC COUNTING PERIODS OF CS
HEART RATE CHANGE FROM BASE (BPM)
10
8
6
4
2
0
-2
-4
-6
-8
-10

magnitude followed by an acceleration during the next few CPs. The response to the light remained accelerated throughout the CS (i.e., biphasic) but the response to the tone returned to baseline levels and then decelerated as the CS continued (i.e., triphasic). When the mean HR changes of the single modality groups to the light and tone were analysed, significant main effects for L/T, +/- and CPs were found (F[1,28] = 9.69, F[1,28] = 7.62 and F[13,364] = 10.02, respectively), as were significant L/T x CPs (F[13,364] = 8.57) and +/- x CPs (F[13,64] = 2.44) interactions. There were no effects due to TBs (see Figure 3).

Visual inspection of Figure 1a suggests that the L/T x CPs interaction is due to the obvious reversal in responding from early CPs to late CPs. In order to examine the +/- x CPs interaction, the data from Figure 1a were redrawn in Figure 2, which shows separately the responses to the subsequently reinforced and non-reinforced light and tone stimuli. Generally, the responses of rats in the L+ and T+ groups were of a smaller magnitude than those of rats in the L- and T- groups.

Combined Modality Groups. The responses to the light and tone of the combined modality groups are graphed in Figure 1b averaged over the +/- and TBs variables. The topographies of these responses are similar to those described for the single groups, i.e., the response to the light is biphasic and the response to the tone is triphasic. Analysis of responding

Figure 2. The mean heart rate responses to the stimuli presented to the four single stimulus modality groups during stimulus pre-exposure trials. These responses were collapsed over trial blocks. The + and - symbols indicate whether these events were reinforced or nonreinforced during subsequent conditioning.

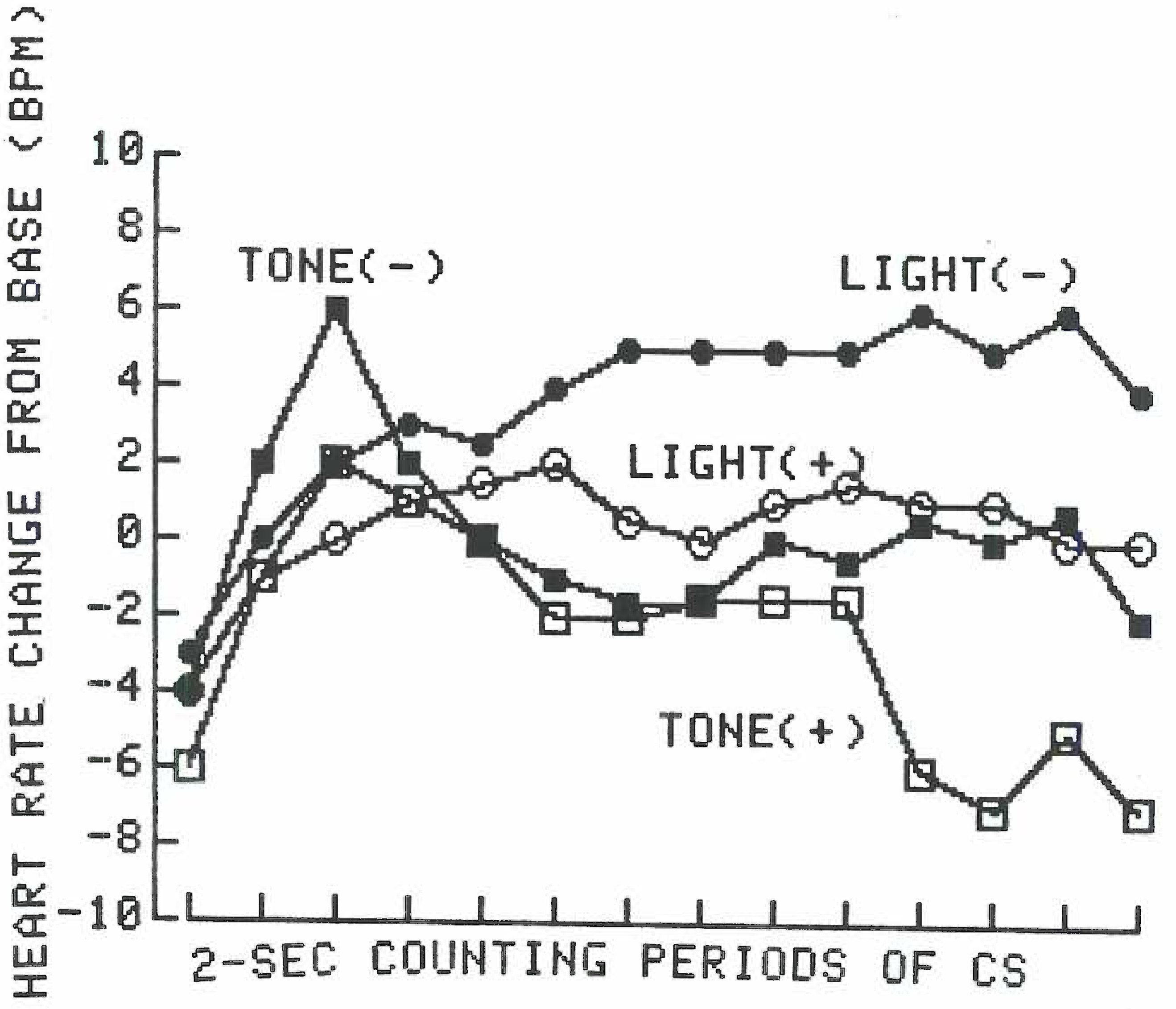
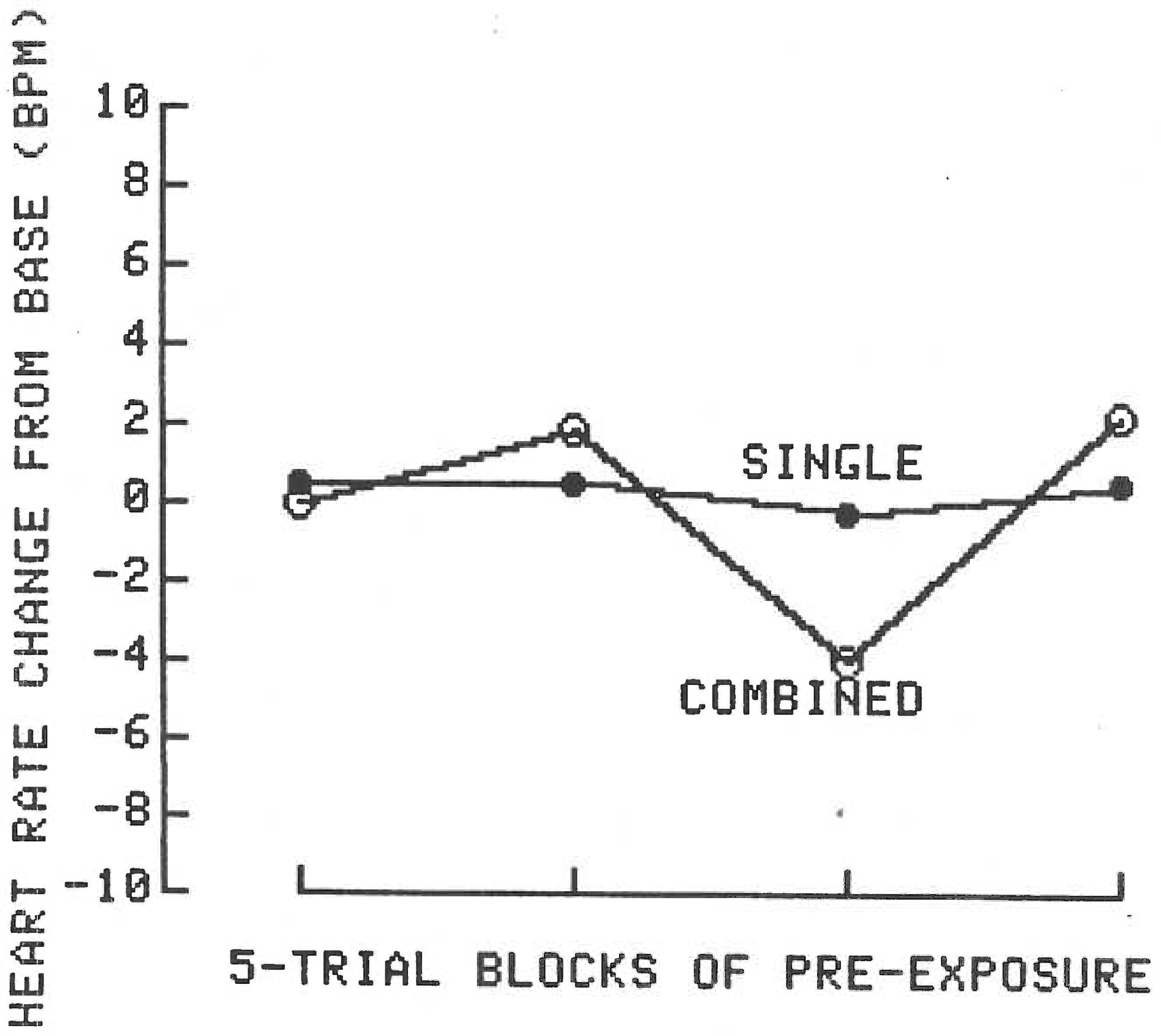

HEART RATE CHANGE FROM BASE (BPM)
10
8
6
4
2
0
-2
-4
-6
-8
-10
2-SEC COUNTING PERIODS OF CS
TONE(-)
LIGHT(-)
LIGHT(+)
TONE(+)

Figure 3. The mean heart rate change scores for the single- and combined-modality groups in each TB of stimulus pre-exposure averaged across S/C, +/- and CPs.


HEART RATE CHANGE FROM BASE (BPM)
10
8
6
4
2
0
-2
-4
-6
-8
-10
SINGLE
COMBINED
5-TRIAL BLOCKS OF PRE-EXPOSURE

in the combined groups revealed significant main effects for TBs and CPs, (F[3,42] = 7.07 and F[13,182] = 2.4, respectively) and significant +/- x TBs x CPs (F[39,546] = 1.64), L+/T+ x +/- x CPs (F[13,182] = 2.88), and L+/T+ x +/- x TBs x CPs (F[39,546] = 2.29) interactions. Separate three-way ANOVAs were run on the data from the animals who were scheduled to receive CS+ presentations and those scheduled to receive CS- presentations during subsequent conditioning sessions. A significant L-/T- x TBs x CPs interaction was found in the analysis of CS(-) (F[39,546] = 2.09) while only a TBs x CPs interaction was found in the analysis of CS(+) (F[39,546] = 1.52). Separate two-way analyses of the "negative" stimuli led to the finding of a significant TBs x CPs interaction (F[39,546] = 2.00) for the L(-) stimulus only. Thus, these analyses revealed TBs x CPs interactions for all but the L+ stimulus, which only showed significant main effects for these two variables. Generally, the interaction appears to be due to an absence of multiphasic responding in the third TB to all but the L+. During the third TB, responding is predominantly deceleratory (monophasic) for the L(-) and tone stimuli. For L(+), the responding in the third TB is also depressed (relative to other TBs) but is still biphasic. At this point in testing, however, there is no difference in treatment between the light presentations (i.e., all stimuli are non-reinforced). The general trial block effect is shown in Figure 3 averaged across the between group variable, +/- and CPs. As described above, response magnitude increased across trial blocks but was depressed in TB 3.

STIMULUS PRE-EXPOSURE: Single Modality vs. Combined Modality

Light Responses. In order to evaluate the effects of the number of stimulus of different modalities presented per session, the responses to the light of the single and combined modality groups were compared. The mean responses of the single and combined groups to the light are already shown in Figs. 1 a and b, respectively. Both responses are biphasic and appear to be of similar magnitudes. Analysis of the mean heart rate change scores during the light CS revealed a significant main effect of CPs (F[13,364] = 13.88) and of TBs (F[3,84] = 5.49). In addition, significant +/- x TBs (F[3,84] = 3.71) and +/- x CPs interactions (F[13,364] = 4.2) were found. There were no effects due to S/C.

To examine the implications of the interactions, the data were first collapsed across S/C and TBs to assess the effects of +/- over CPs. These effects can be seen in Figure 2. The response to the subsequently non-reinforced stimulus was generally of greater magnitude than that to the subsequently reinforced stimulus. The second interaction was examined by collapsing across the S/C and CPs variables and graphing these means in Figure 4. The same pattern of response magnitude across TBs can be seen as that shown in

Figure 4. The mean heart rate change scores to the light CS during stimulus pre-exposures of subjects in Group L+ and L- averaged over S/C and CPs variables.

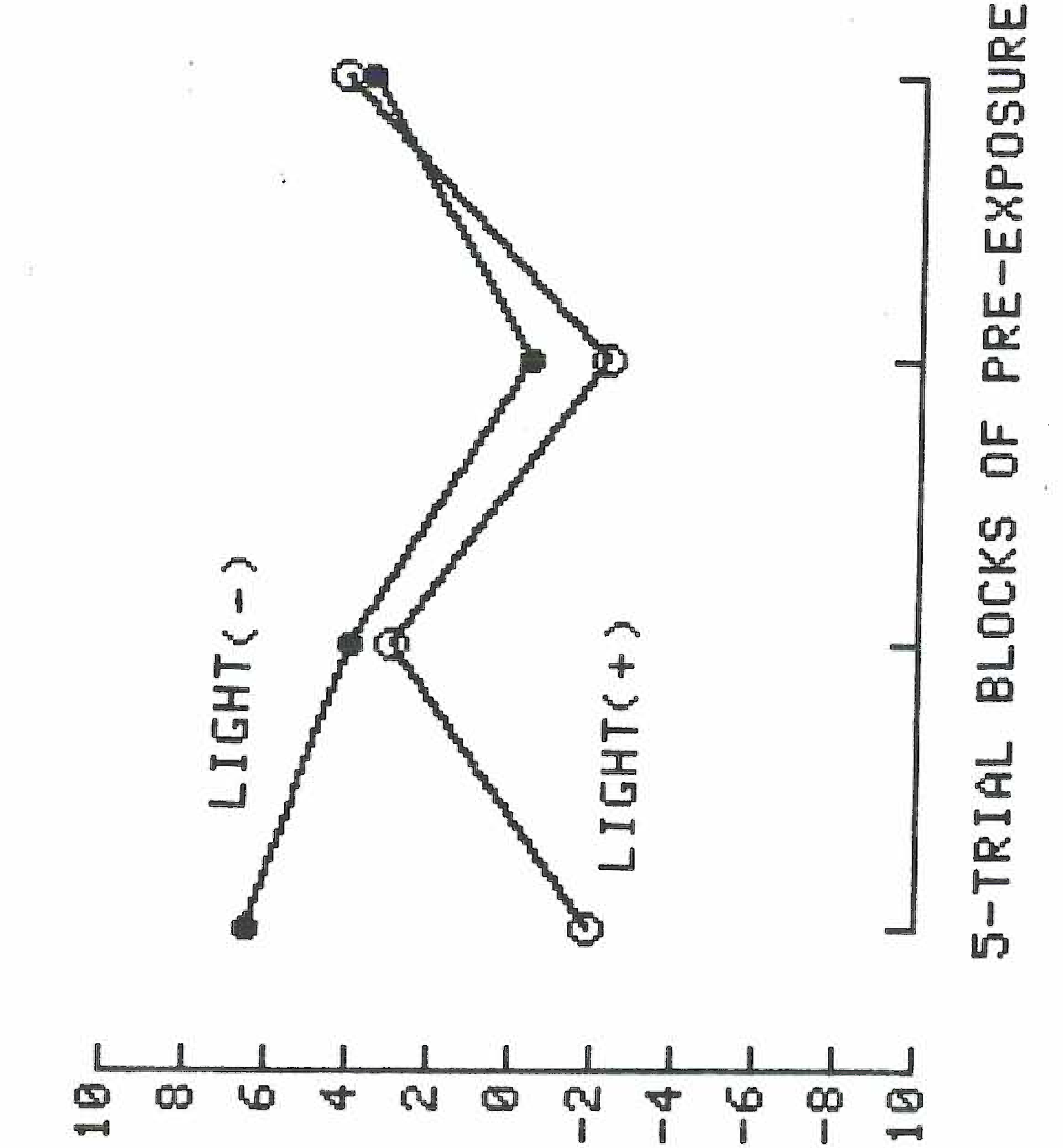

HEART RATE CHANGE FROM BASE (BPM)
10
8
6
4
2
0
-2
-4
-6
-8
-10
LIGHT(-)
LIGHT(+)
5-TRIAL BLOCKS OF PRE-EXPOSURE

Figure 3, except that accelerations were elevated for L- during the first TB. Therefore, it appears as if response magnitude generally decreases with repeated pre-exposure to L- which might be interpreted as habituation. However, it is also likely that the first point of the L(-) data is abberrant due to sampling error since the effect is not seen to L(+) and again, at this point, there was no difference in reinforcement schedules of these stimuli. In support of the latter hypothesis, response magnitude increases during the fourth TB, which is not characteristic of a habituation effect.

Tone Responses. The responses of the single and combined groups to the tone CS are shown in Figures 1 a and b. Both responses were triphasic and appear to be similar in magnitude. Analysis of these responses showed significant effects for CPs (_F_[13,364] = 24.97) and TBs (_F_[3,84] = 3.01). The TBs effect for tone is similar to that graphed in Figure 3 (which combines light and tone responses), showing response magnitude depression in TB 3.

STIMULUS PRE-EXPOSURE: Summary

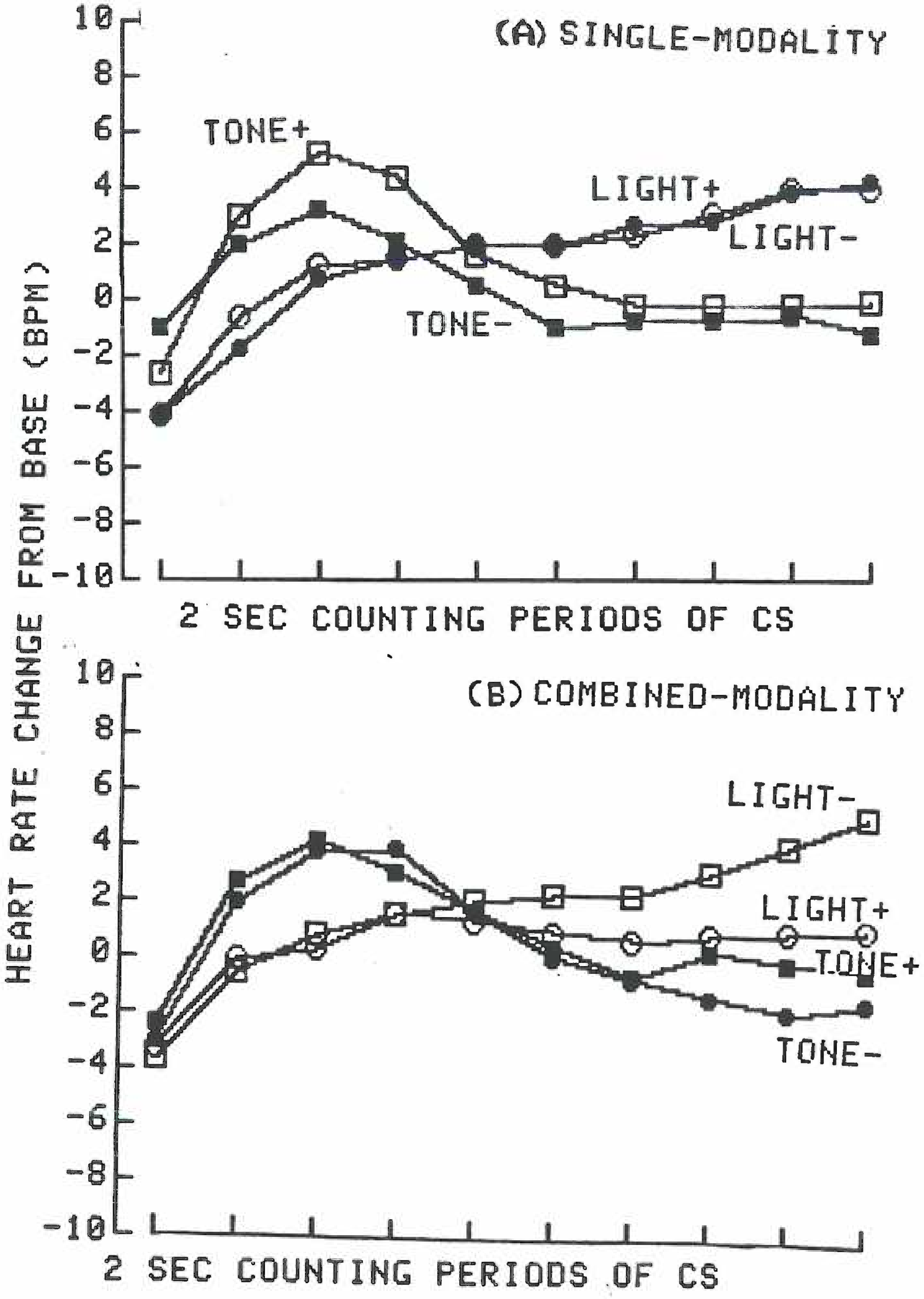
Analyses of the unconditioned responses to the CSs consistently revealed a characteristic biphasic response to the light and a triphasic response to the tone, independent of the number of stimulus modalities presented per session. It did not appear as if these patterns diminished with trials (characteristic of habituation) although a decrement in responding was seen for almost all groups in the third trial block. This was the first trial block of the second day of testing, therefore, the decrement may be due to the intersession interval between the first day of pre-exposure and the second. Significant +/- interactions were found for some of the comparisons but were considered to be due to sampling error since there was no difference in reinforcement schedules during pre-exposure sessions. These effects will be considered again in the analyses of +/- differences in conditioning sessions.

CONDITIONING: Light vs. Tone

Single-Modality Groups The mean heart rate responses of the single-modality groups to the reinforced and nonreinforced lights and tones over counting periods are shown in Figure 5a averaged over all conditioning trials. The same general differences in responding between light and tone observed during stimulus pre-exposure are still apparent with maximal accelerations of about 4 bpm occurring during the early part of the tone and the last part of the light. Analysis of the responses for the single modality groups revealed a significant CPs main effect ($F[9,252] = 24.66$) and significant L/T x CPs ($F[9,252] = 27.0$) and L/T x +/- x TBs x CPs interactions ($F[45,1210] = 1.42$).

Separate threeway ANOVAs run on the data from the light and tone groups revealed a significant +/- x TBs x CPs interaction for the light groups ($F[45,620] = 1.46$) and only a TBs x CPs ineraction for the tone groups ($F[45,590] =$

Figure 5. The mean heart rate responses to reinforced (+) and nonreinforced (−) lights and tones presented to the (a) single-modality groups and (b) combined-modality groups during conditioning sessions.



(A) SINGLE-MODALITY

(B) COMBINED-MODALITY

1.38). Separate within-group analyses on the L+ and L- groups revealed a significant TBs x CPs interaction for the L- group only (F[45,620] = 1.44) and no significant TB effects on responding to L+. Thus, all single modality groups demonstrated a TBs x CPs interaction except for the L+ group. There was no overall direction to these interactions, however, with responding varying in an unsystematic way over trial blocks. Therefore, when comparing responses to the L+ and L-, in some TBs the response to the CS- was of a greater magnitude and in other TBs the response to CS+ was of greater magnitude. There were no significant main effects for TBs found in any of the followup analyses.

Combined-Modality Groups. The responses of the combined groups to the L+, T+, L- and T- are graphed in Figure 5b averaged over trial blocks. These responses resemble the characteristic responses to the light and tone as described above. There appeared to be a difference in the magnitudes of the accelerative responses to L+ and L- stimuli near the end of the CS-US interval, with the response to L- appearing greater and no difference between the magnitudes or directions of responses to the T+ and T-. Significant effects revealed in the analysis of the responses of the combined groups included a CPs main effect (F[9,126] = 24.67), and L+/T+ x CPs (F[9,126] = 2.76) and L+/T+ x +/- x CPs (F[9,126] = 21.17) interactions. This last interaction is due to a reversal in the direction of the heart rate response from an acceleration to a deceleration to both tone CSs as opposed to a sustained acceleration to both light CSs, and can be seen in Figure 5b. The significance of the L+/L- difference will be discussed in the next section, where it is directly analyzed.

CONDITIONING: Single vs. Combined Groups

Light Responses. The responses to the light in the single and the combined groups are shown in Figure 5a and b and appear for the most part to be similar. The general pattern of responding to the light was biphasic, but as mentioned previously, in the combined modality groups, the response to the L- was of a greater magnitude than the response to the L+ at the end of the CS-US interval. Analysis of the responses yielded a significant main effect for CPs (F[9,252] = 49.55), and significant +/- x CPs (F[9,252] = 2.03) and S/C x +/- x TBs x CPs (F[45,1190] = 1.53) interactions. Separate three-way ANOVAs comparing the two single groups or the two combined groups revealed a significant +/- x TBs x CPs interaction in the single groups only (F[45,620] = 1.46). Two-way within-group ANOVAs of the single groups revealed a significant TBs x CPs interaction in the L- group (F[45,620] = 1.44) but no TB effects in the L+ group. The TBs x CPs interaction in the L- groups seems attributable to the same unsystematic variation of response magnitude over TBs as described earlier. This effect of TBs is not what one might expect of an acquisition curve (e.g., an gradual increase or decrease in response magnitude over trials).

When the S/C differences were compared using separate three-way ANOVAs on the L+ and L- responses, the S/C x TBs x CPs interaction was found to be significant (_F_[45,620] = 1.32) for the L- stimuli only. However, when this interaction was followed by the two-way within ANOVAs on the separate groups (i.e., single vs. combined), no significant effects were found. It was therefore concluded that the variation of response magnitude over TBs and CPs shown to be significant for the single-modality L- stimulus was not a reliable effect.

The followup analyses also revealed a +/- x CPs interaction for the combined modality groups (_F_[9,126] = 2.71) but not for the single-modality stimuli. This supports the difference seen between the L+ and L- groups in Figure 5b. This is the same difference seen during pre-exposure trials that was attributed to nonassociative factors.

Tone _CRs_. The responses to the reinforced and non-reinforced tones averaged over all 60 trials are shown for the single group and the combined group in Figure 5a and b. The response to the tone was still triphasic and appeared similar in both groups. Significant effects found in the analysis were for CPs (_F_[9,252] = 46.39) and S/C x TBs x CPs (_F_[45,1160)=1.53). The interaction was evaluated by two separate three-way ANOVAs on the data from the single groups and the data from the combined groups. A significant TBs x CPs effect was found only for the single modality groups

(F[45,590] = 1.38). As described earlier, the magnitude of responding varied unsystematically over TBs and CPs in these groups.

CONDITIONING: Summary

The characteristic response patterns to the light and tone were still present during all conditioning trials. The only differences in responding between paired and unpaired stimuli that occurred consistently were larger accelerations to the L- stimulus of the combined group as compared to the L+ near the end of the CS-US interval. Due to the existence of a similar +/- difference in responding to the light pre-exposure trials, this was not considered evidence of conditioning. When the mean heart rate responses to the light and the tone are graphed in Figure 6 as a function of TBs (averaged over S/C and CPs), the absence of an effect of paired (+) or unpaired (-) trials on responding is emphasized.

EXTINCTION: Tone vs Light

Single Modality Groups.

The differences in responding of the single-modality groups to the reinforced and non-reinforced lights and tones during extinction are shown in Fig. 7a averaged over S/C and TBs. The responses to both lights were biphasic , however, the response to L(+) reached a greater magnitude of acceleration as compared to that L(-) by the end of the ISI. The tone responses were both triphasic and there appeared to be no difference in responding between the T(+) and T(-). An analysis of the data from the single

Figure 6. The mean responses to the reinforced (+) and non-reinforced (−) light and tone across 10-trial blocks of conditioning. The responses were averaged across S/C and CPs variables.

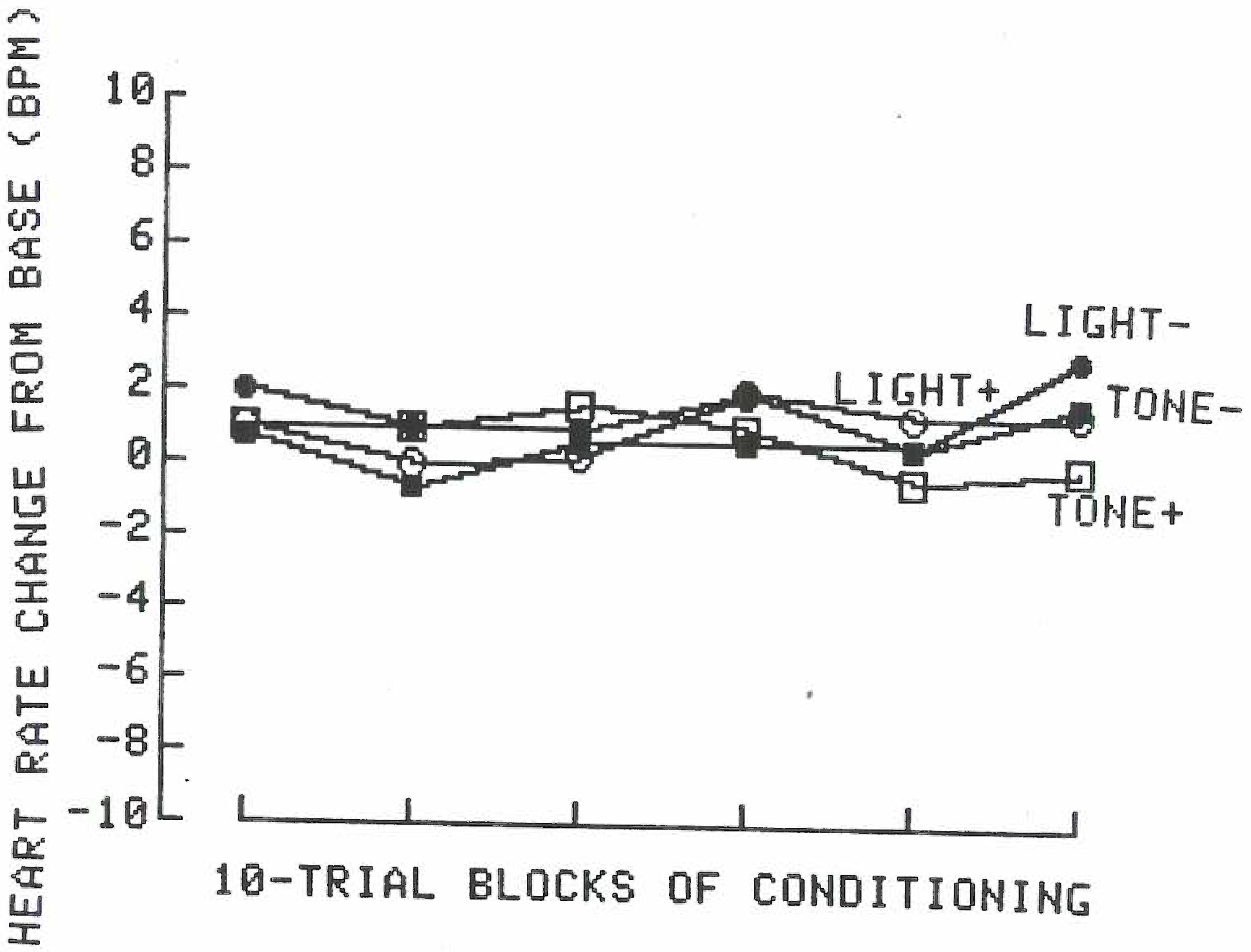
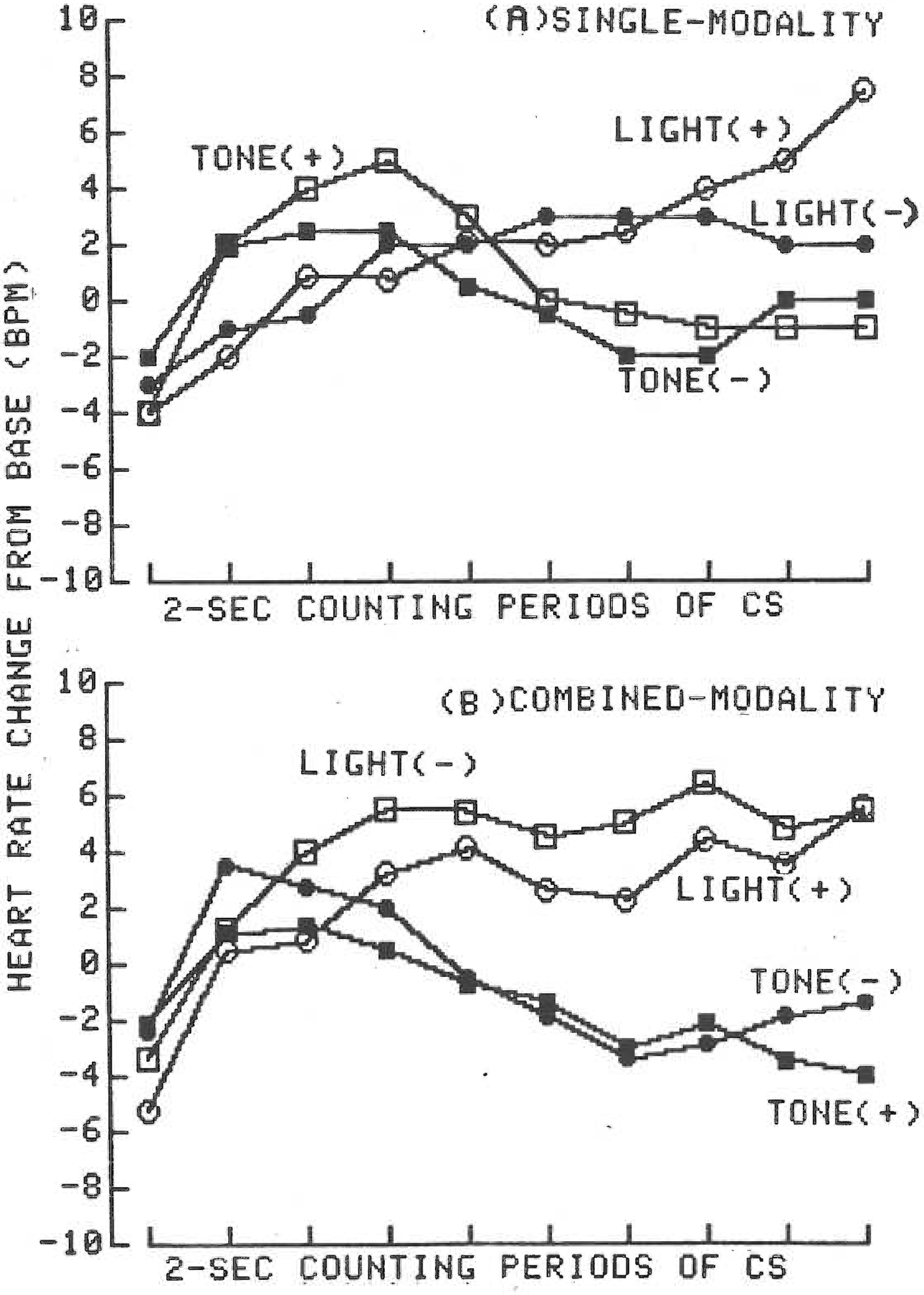

HEART RATE CHANGE FROM BASE (BPM)
10
8
6
4
2
0
-2
-4
-6
-8
-10
10-TRIAL BLOCKS OF CONDITIONING
LIGHT-
LIGHT+
TONE-
TONE+

Figure 7. The mean responses to the previously reinforced (+) and nonreinforced (−) light and tone of (a) the single-modality groups and (b) the combined-modality groups during extinction sessions.


HEART RATE CHANGE FROM BASE (BPM)
(A)SINGLE-MODALITY
10
8
6
4
2
0
-2
-4
-6
-8
-10
TONE(+)
LIGHT(+)
LIGHT(-)
TONE(-)
2-SEC COUNTING PERIODS OF CS
(B)COMBINED-MODALITY
10
8
6
4
2
0
-2
-4
-6
-8
-10
LIGHT(-)
LIGHT(+)
TONE(-)
TONE(+)
2-SEC COUNTING PERIODS OF CS

modality groups during extinction revealed a significant L/T x +/- x CPs interaction (*F*[9,252] = 2.15). Other significant effects were for CPs (*F*[9,252] = 15.4) and for L/T x CPs (*F*[9,252] = 14.04). Separate three-way ANOVAs on the light and tone responses revealed a significant +/- x CPs interaction for the light responses (*F*[9,126] = 1.98) but not for the tone responses. There were no significant effects involving the +/- variable for the tone responses. The difference in magnitude of acceleration between L(+) and L(-) was not seen in stimulus pre-exposure or conditioning. There were no effects of TBs.

Combined Modality Groups. The responses to the L(+), L(-), T(+), and T(-) are graphed in Fig. 7b. Modality-specific responding is still present. Responses to the previously unreinforced stimuli appear to be of greater magnitude than those to the reinforced stimuli. This is consistent with differences seen during the stimulus pre-exposure and conditioning sessions. The statistics for these groups included significant effects for TBs (*F*[3,42] = 3.33), CPs (*F*[9,126] = 8.13), L+/T+ x +/- x CPs (*F*[9,126] = 7.86) and TBs x CPs (*F*[27,378] = 2.12). The three-way interaction is explainable by visual inspection of the figure, i.e., the direction of responding to the tone reverses from an acceleration to a deceleration during the latter part of the ISI, while the response to the light remains accelerated throughout the ISI, without regard to the +/- variable.

The effect of trial blocks on the responses over CPs was a general increase in response magnitude in the fourth trial block during all CPs except the first and second. This does not seem to indicate an effect of extinction since it does not oppose any of the few response changes due to reinforcement schedule that occurred during conditioning trials (none of which were found to be statistically significant).

EXTINCTION: Single vs Combined

Light Responses. The response to the light during extinction sessions is shown in Figure 7a for the single and in Figure 7b for the combined groups. The biphasic response was present in both the single and combined groups, consisting of a brief deceleration of about 4 bpm in the first CP followed by a gradually increasing acceleration that reached a maximum of about 5 bpm by the tenth CP. The ANOVA performed on the light CR data from Groups L+, L-, L+/T- and T+/L- revealed only a significant main effect for CPs ($F[9,252] = 17.58$). The differences between L(+) and L(-) that was seen in the extinction analysis of the single-modality groups was not present in this analysis (which included both single and combined modality groups). This inconsistency suggests that any difference between L+ and L- responding was marginal. The failure to get a TBs effect implies that no extinction or habituation effects were present. There was no effect of the S/C variable.

Tone Responses. The responses to the tone during extinction have been shown in Figures 7a and b. The triphasic response to the tone was still generally present. An analysis performed on the tone responses from Groups T+, T-, T+/L- and L+/T- revealed a CPs main effect (F[9,252] = 22.75) and significant interactions for S/C x CPs (F[9,252] = 2.03), S/C x TBs (F[3,84] = 3.44), S/C x TBs x CPs (F[27,756] = 1.9) and S/C x +/- x TBs (F[3,84] = 3.02). Separate three-way ANOVAs on the single and the combined groups revealed a significant TBs x CPs interaction for the combined groups (F[27,378] = 2.32) and no effects involving TBs for the single groups. As described above and shown in Figure 8 (for both stimuli), the response magnitude to the tone is elevated in the fourth TB for all CPs except the first and the second.

Evaluation of the S/C x +/- x TBs interaction using separate three-way ANOVAs on the T(+) and T(-) responses revealed a significant S/C x TBs interaction for the T+ stimuli only (F[3.42] = 3.76). However, none of the TBs main effects were significant in any of the within-group ANOVAs. The difference in response magnitudes over trial blocks is shown for the T+ responses in Figure 9a and for the T- responses in Figure 9b. It appears as if the difference in response magnitudes during the third TB is the reason for the significant S/C x TBs interaction. Due to the absence of significant TBs effects on either of these responses, it is likely that the difference in the third TB is not a reliable

Figure 8. The mean responses to the CS of the combined modality groups for each TB (1-4) during extinction sessions. Responses were averaged over the L/T and +/- variables.

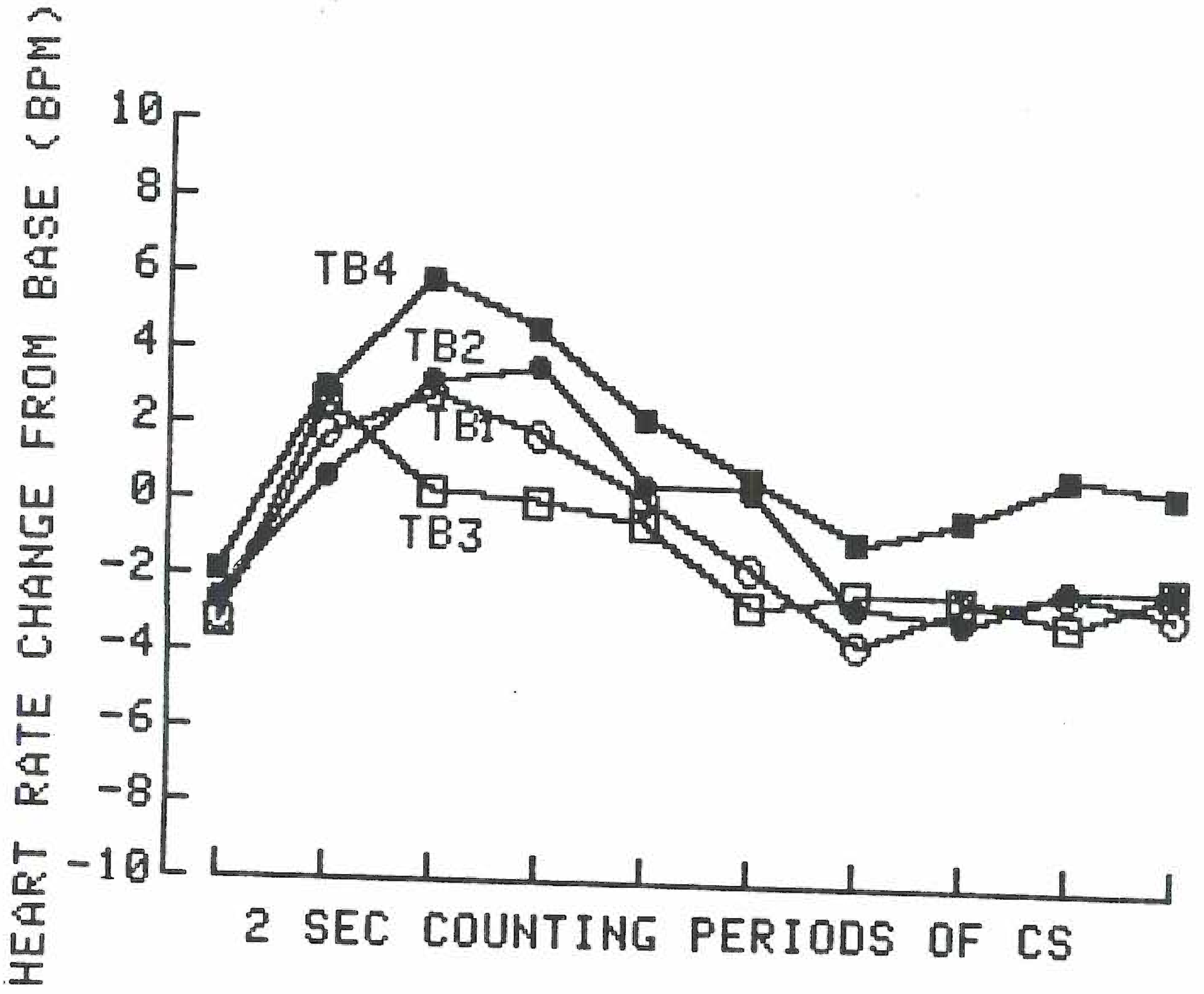
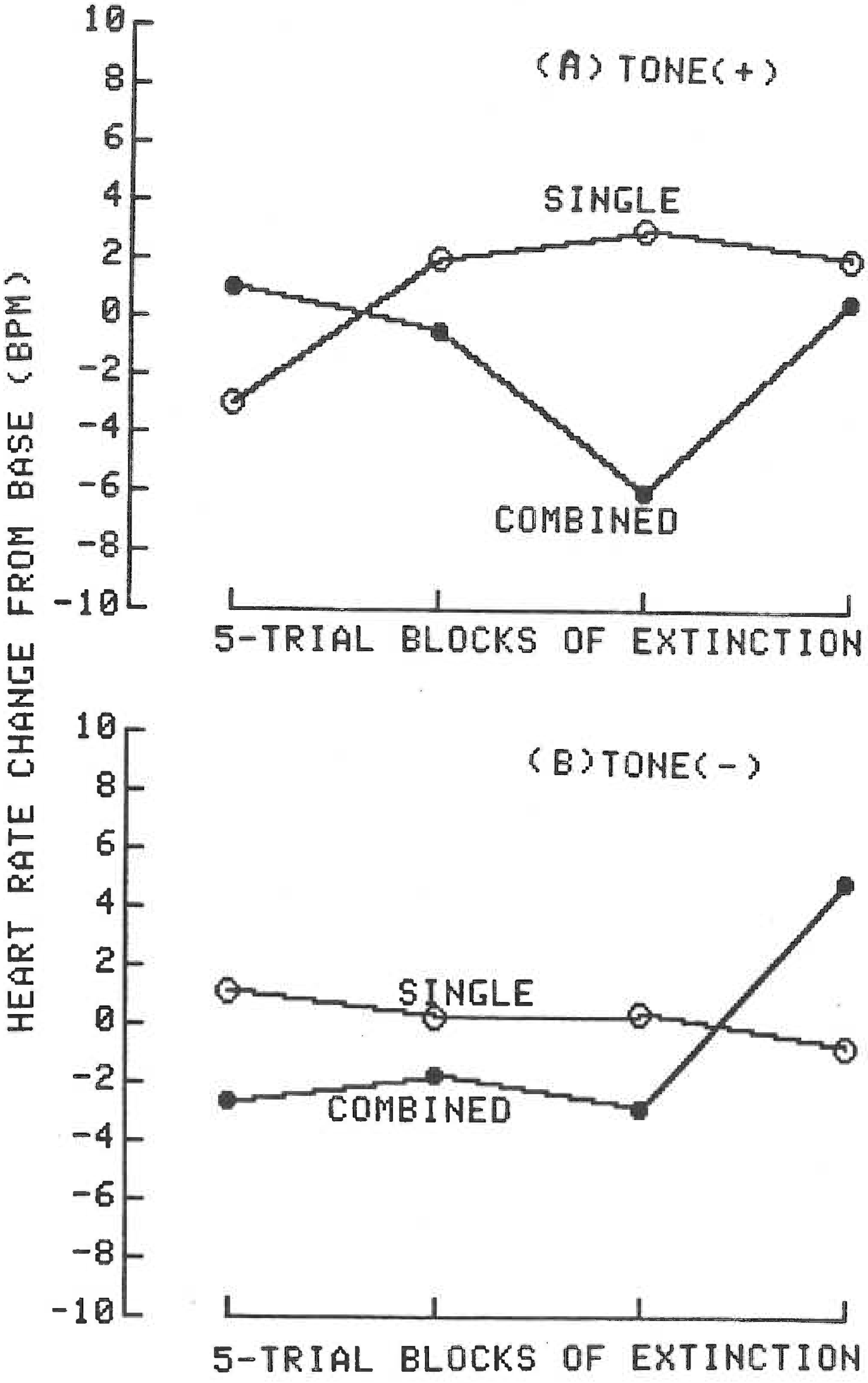

HEART RATE CHANGE FROM BASE (BPM)
10
8
6
4
2
0
-2
-4
-6
-8
-10
TB4
TB2
TB1
TB3
2 SEC COUNTING PERIODS OF CS

Figure 9. The mean heart rate response in the single- and combined-modality groups to the tone CSs during extinction trials for (a) the previously reinforced (+) tones and (b) the previously non-reinforced (-) tones. Responses were averaged over CPs.


HEART RATE CHANGE FROM BASE (BPM)
(A) TONE(+)
SINGLE
COMBINED
10
8
6
4
2
0
-2
-4
-6
-8
-10
5-TRIAL BLOCKS OF EXTINCTION
(B) TONE(-)
SINGLE
COMBINED
10
8
6
4
2
0
-2
-4
-6
-8
-10
5-TRIAL BLOCKS OF EXTINCTION

one.

EXTINCTION: Summary

Modality-specific responding similar to that described for stimulus pre-exposure and conditioning was also present during extinction trials. There did not seem to be a decremental effect of extinction on response magnitude as might be expected. Instead, response magnitude was not seen to change over TBs, except for a depression in the third TB of the T+ response of the combined modality groups. This depression was not revealed by a TBs main effect and therefore, was not considered to be a reliable difference. There was no effect of TBs in the single modality groups.

UNCONDITIONED RESPONSES

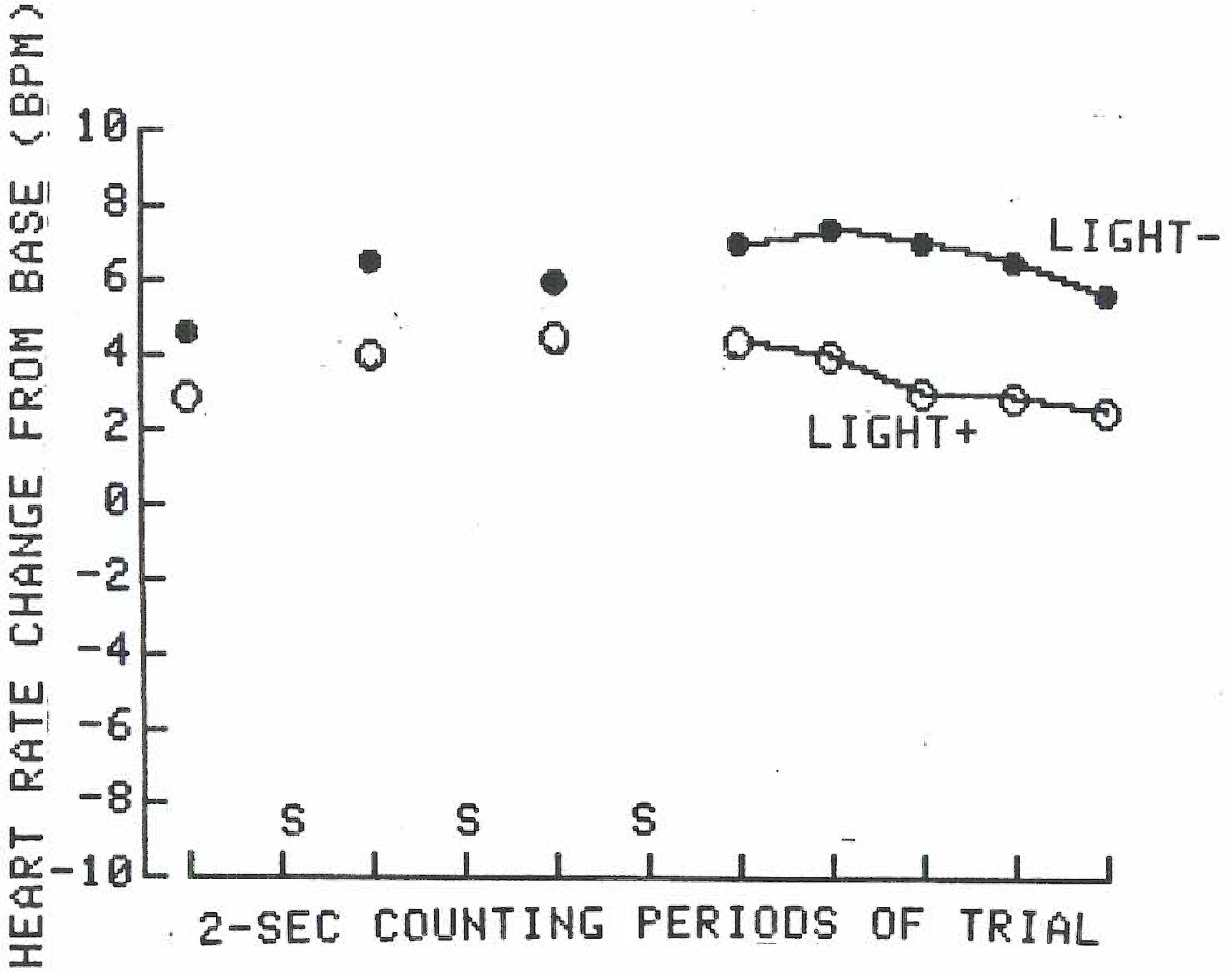
Responses to the shock sequence were measured during the last 22 sec of each conditioning trial. This included the 2-sec CP immediately prior to shock onset (already included in the above analyses as the last period of the CR), two 2-sec intershock intervals and five 2-sec CPs after the last CP during which a shock occurred. The three 2-sec CPs during which shock pulses occurred were not monitored and for comparison these same CPs were omitted from all nonreinforced trial records. The comparison of primary interest was between responding on reinforced trials and responding on non-reinforced trials to observe any changes in responding during the two stimulus modalities induced by shock. Also of interest were differences between responding to shock alone and responding to the combination of CS and shock. Therefore, comparisons were made between responses to shock during paired CS-US trials and responses to shock during explicitly US-alone presentations.

Thus, separate four-way ANOVAs were run on the data from the last 22 sec of the tone presentations and of the light presentations using between group variables of modality number (S/C) and reinforcement schedule (+/-) and within group variables of TBs and CPs. A separate four-way ANOVA was run to compare responding during the shock alone with responding during the shock presented with a tone or light CS. The between group variables were stimulus modality present during shock (L/T/O) and a dummy variable (second stimulus type) that was used to keep the six groups separate. The within group variables were TBs and CPs.

URs: Reinforced vs Non-reinforced

Light. Figure 10 plots the HR changes to reinforced and nonreinforced lights during the last 22 sec of a trial averaged over S/C and TBs. The response to the light, both with and without shock, was predominantly acceleratory. However, responding during the light plus shock was depressed when compared to that during the light alone. The four-way ANOVA found significant main effects for +/- (*F*[1,28] = 4.67), TBs (*F*[5,140] = 2.59) and CPs (*F*[7,196] = 4.36). It is important to note that the response to L+ during the first 20 sec of conditioning trials was also found to be of a

Figure 10. The mean heart rate response to the US (S = 2-sec counting period during which shock occurred for the reinforced stimuli only) presented during the reinforced light (+) as compared to the response to the non-reinforced light (-) during the last 22 sec of conditioning trials. These data are averaged over the S/C and TBs variables.


HEART RATE CHANGE FROM BASE (BPM)
10
8
6
4
2
0
-2
-4
-6
-8
-10
S
S
S
2-SEC COUNTING PERIODS OF TRIAL
LIGHT-
LIGHT+

smaller magnitude than the response to L-, especially during the last portion of the ISI. It is therefore likely that the difference shown in Figure 10 is due to existing levels of heart rate at the time of shock delivery rather than direct effects of the shock.

The effect TBs did not seem to follow a systematic pattern but instead, the magnitude of the accelerations to both L+ and L- varied in what appeared to be a random fashion over TBs. Such an effect was also present during the first 20 sec of the trial but only for the L- groups. There were no effects on responding due to the S/C variable.

Tone. Figure 11 shows the mean heart rate change scores to the tone with and without shock averaged over S/C and TBs variables. There appears to be no differences between the magnitudes or the directions of the two responses. The four-way ANOVA showed no significant main effects or interactions. Therefore, there was no difference in the response to the tone with and without the US present.

URs: Shock plus CS vs Shock Alone

The responses to shock alone and shock during the light or the tone are graphed in Figure 12 averaged across the between group dummy variable and TBs. No differences in responding during shock alone and shock plus the other stimulus events was apparent. Significant effects revealed by the analysis were for L/T/O (F[2,42] = 6.14), TBs (F[5,210] = 2.43) and CPs (F[7,294] = 5.13). When

Figure 11. The mean response to the US (S = 2-sec counting period during which shock occurred for the reinforced stimuli only) presented during the reinforced tone (+) as compared to the response to the non-reinforced tone (-) during the last 22 sec of conditioning trials. These data were averaged over the S/C and TBs variables.

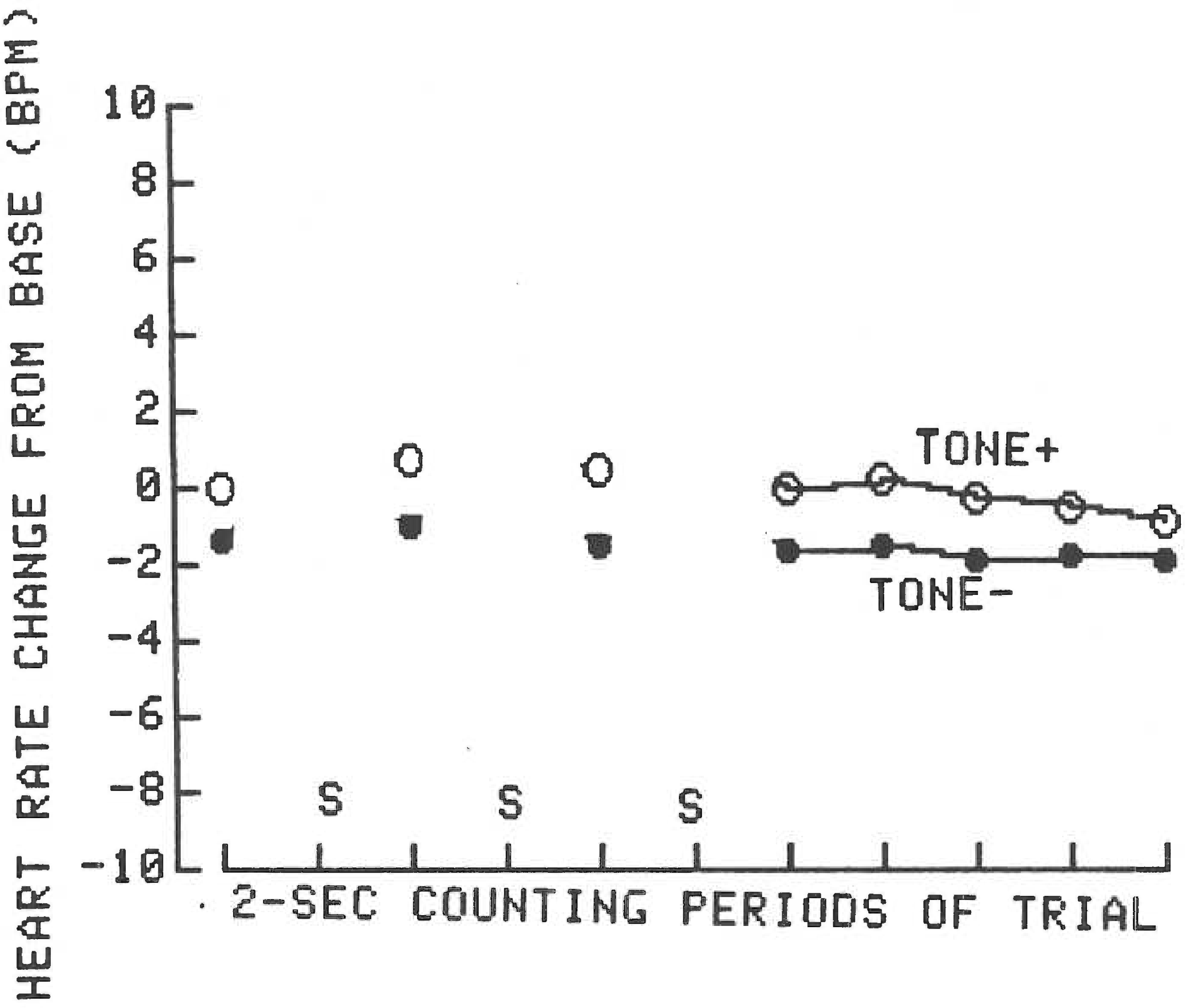
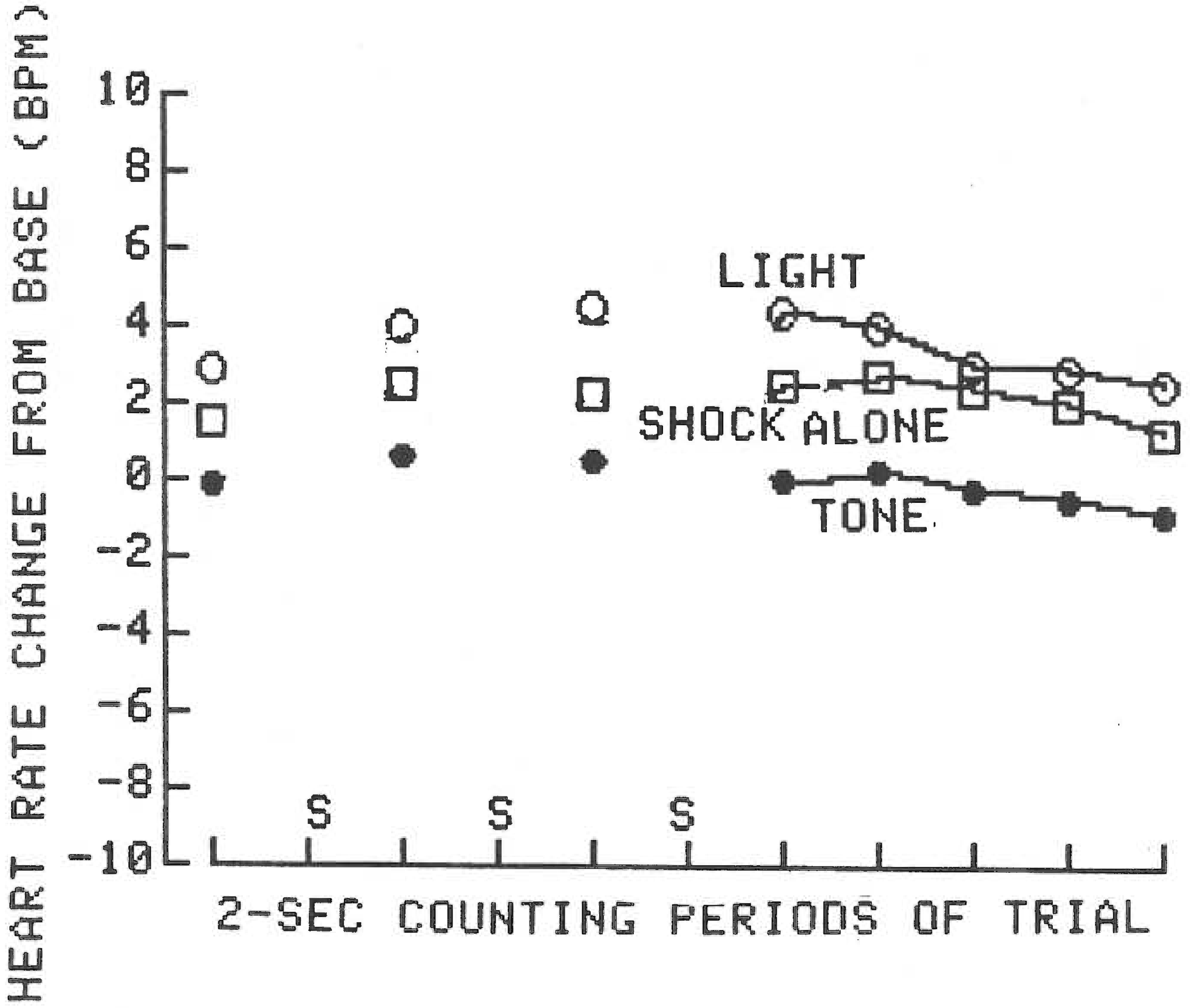

HEART RATE CHANGE FROM BASE (BPM)
10
8
6
4
2
0
-2
-4
-6
-8
-10
S
S
S
TONE+
TONE-
2-SEC COUNTING PERIODS OF TRIAL

Figure 12. The mean responses to the shock (S) alone, the shock with light (LIGHT+) and the shock with tone (TONE+) during the last 22 sec of extinction trials. These data were averaged over the S/C and TBs variables.


HEART RATE CHANGE FROM BASE (BPM)
10
8
6
4
2
0
-2
-4
-6
-8
-10
S
S
S
2-SEC COUNTING PERIODS OF TRIAL
LIGHT
SHOCK ALONE
TONE

comparisons were made between the means of the three responses using the Neuman-Keuls method, the only significant difference found was between the response to the shock during the tone and the shock during the light (Cn-k[3,768] = 2.5). The response to shock alone was not significantly different from the response to shock combined with the light or the tone.

The mean heart rate change scores during the shock alone were averaged across all within-group variables for each subject and these means were compared with zero using a student's T test. There was no significance difference found between the heart rate change scores during and after the shock and zero. It was therefore concluded that the shock alone did not elicit a noticeable heart rate response during or after US administration.

URs: Summary

Since the only significant difference between responses during shock and no shock were during the light CS and those differences were also present before shock onset, and the only significant differences between shock during the CS events and shock alone were attributed to the CS events, the ability of the US to elicit a noticeable heart rate change was tested. When no difference was found between the heart rate changes during and after the US and zero, it was concluded that the US employed in this study did not elicit a reliable UR.

BASELINE HEART RATE

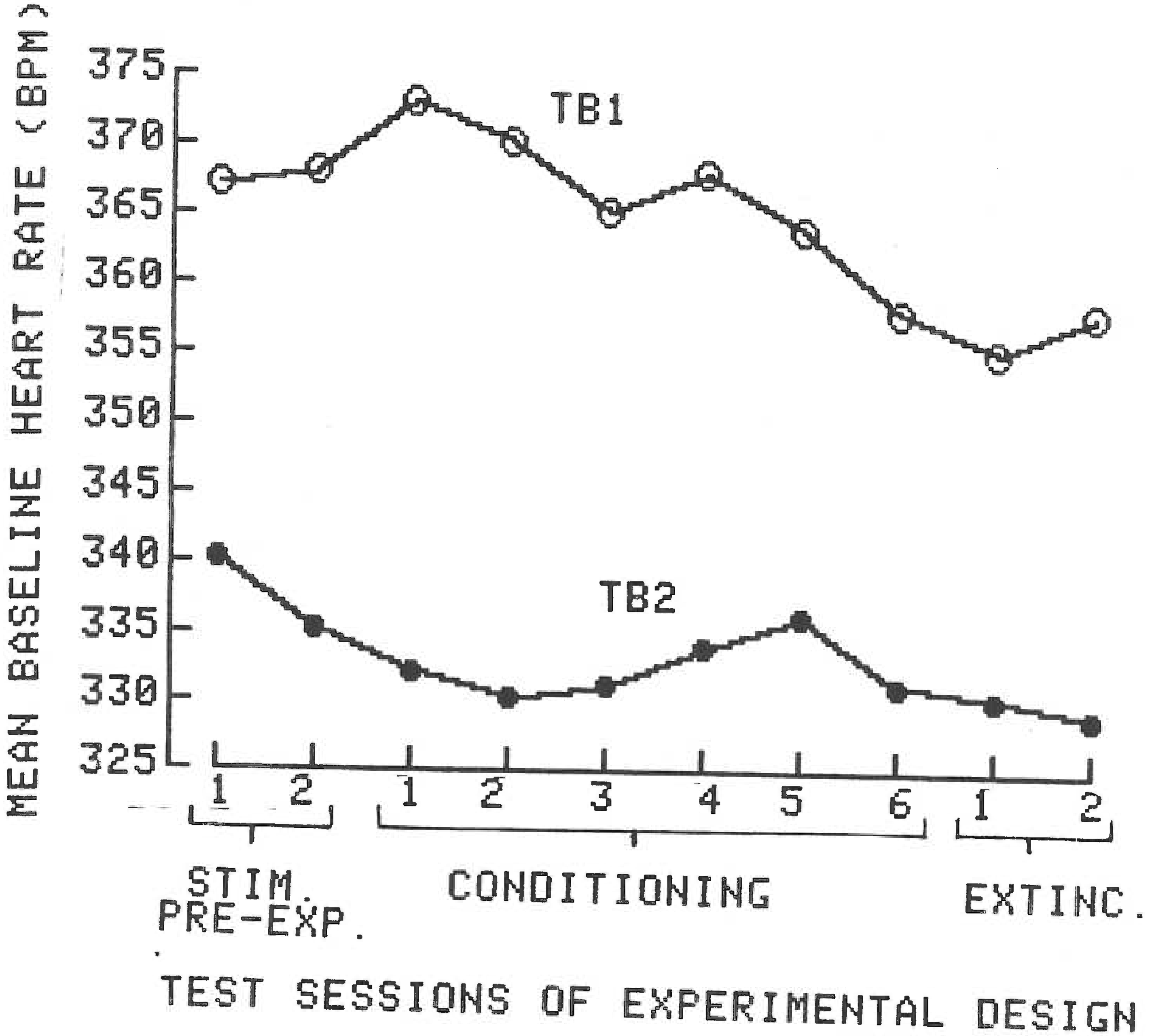
Mean baseline heart rates computed from the 10-sec pre-CS period before each trial were averaged over all trials during sessions halves (i.e., TBs) per group and analyzed with a three-way ANOVA using groups as a between groups variable and days and TBs as the within groups variables. These comparisons were made in order to assess differences between pre-trial baseline heart rates between groups, between sessions and within sessions.

Mean baseline scores are graphed in Figure 13 averaged over the groups variable (there were no reliable differences between groups). It appears as if baseline heart rate generally decreased over days of testing and was greatly reduced during the second TB of each day. The ANOVA revealed significant main effects for days (F[9,373] = 3.37) and TBs (F[1,42] = 372.96) and a significant days x TBs interaction (F[9,366] = 3.4). Examination of the figure suggests that this interaction is due to the fact that differences between TB 1 and TB 2 were greatest during the first few blocks of conditioning, but later diminished. Since the baseline levels of responding were equal between groups, the differences seen in responding cannot be attributed to differences in heart rate that existed before trials began. In addition, the strong TBs effect on baseline does not appear to have been reflected in the difference scores analyzed in any phase.

Discussion

The lack of consistent differences between responding to paired and unpaired stimuli suggests that conditioned

Figure 13. Mean baseline responding during the first and second trial blocks (TB1 and TB2) of each test session averaged across groups.


MEAN BASELINE HEART RATE (BPM)
375
370
365
360
355
350
345
340
335
330
325
TB1
TB2
1
2
1
2
3
4
5
6
1
2
STIM.
PRE-EXP.
CONDITIONING
EXTINC.
TEST SESSIONS OF EXPERIMENTAL DESIGN

responses did not develop during the 60 conditioning trials. Although some differences between responses to CS+ and CS- were seen during the light presentations to single modality groups, these differences also existed during stimulus pre-exposure trials before reinforcement schedules were manipulated. The absence of CRs during conditioning trials precluded the possibility that any response changes seen during extinction trials were due to the occurrence of spontaneous recovery or extinction.

Consistent distinctively shaped heart rate responses to the visual and auditory stimuli used in the experiment were described that did not diminish in magnitude (habituate) after 100 exposures. The response to the tone CS throughout stimulus pre-exposure, conditioning and extinction sessions was always a triphasic inverted U-shaped response that began with an initial small deceleration. Responding then quickly changed to a larger acceleration that gradually returned back to deceleratory levels before the end of the measurement period. In contrast, the response to the light CS was distinctively biphasic, starting with an initial small deceleration which then changed gradually to an acceleration. The maximum acceleration generally occurred at the end of the measurement period.

According to Sokolov (1963), these responses would not be considered ORs, since they were specific to stimulus quality and did not habituate. Instead, he and Graham and

Clifton (1966) would have considered them primarily defensive reactions due to their acceleratory nature during most of the measurement period. The initial deceleration in the responses to both light and tone might have been considered an OR because it was not stimulus specific (although it also did not habituate), but the longer latency deceleration present during the latter part of the tone could not be described as either an OR or a defense response according to Sokolov's and Graham and Clifton's definitions.

The modality-specific, multiphasic responses that were recorded in this experiment, more closely fit Wilson's (1969) definition of spontaneous responses that are specific to the quality and intensity of the eliciting stimulus and have a slow rate of habituation. The response topography to the tone corresponded with Wilson's description of an inverted U-shaped spontaneous cardiac response to a tone in humans. The response topography to the light resembled the biphasic descriptions provided by Cohen and MacDonald (1971), Porges, Stamps and Walters (1974) and Smith and Stebbins (1965) when measured over the same time interval. These responses did not habituate and at times included more transient components (e.g., depressions in response magnitude during the third TB of stimulus pre-exposure) that may have indicated responding to the novelty of the situation (e.g., first return to the experimental situation), although such responses certainly were not consistently present to all stimuli in all groups.

There was no effect due to presenting one or two stimuli of different modalities per session on heart rate responses at any time during testing. This is not consistent with the report of Powell et al. (1971) who found differences in the direction and magnitude of responses to CSs that were presented during a test session with other CSs of either the same or a different modality. However, they used two stimuli of the same modality (e.g., two frequencies of tone) as CS+ and CS- for their single-modality groups, while in this experiment only one stimulus per modality was used and the control for conditioning was included as a between group variable. This difference in experimental procedure complicates interpretations of the difference in results but it is possible that the different response topographies developed during conditioning as they suggested and not because of modality-specific spontaneous responses. If, as proposed by Powell et al (1971), accelerative or decelerative CRs occur due to differences in the occurrence of the first discriminatory response, then when no discriminatory conditioning occurs (as in this experiment), it follows that no differences in CR topography would occur.

This is unfortunate since in order to propose any causal involvement of spontaneous responses in the development of conditioned responses, one would have to manipulate the spontaneous response and then observe the developing conditioned response for effects caused by such manipulations.

If a single vs combined modality paradigm is only effective in manipulating the topography of CRs without affecting the spontaneous response, it would no longer be a method of choice in demonstrating the effects of the spontaneous response on CR development. In addition, since the CS-US contingencies of this experiment were not successful in establishing conditioned responses, conclusions about the determination of CR topography by the spontaneous response could not be made even if spontaneous response manipulations had occurred.

An analysis of the unconditioned responses to the US revealed no significant heart rate changes that could be attributed to the shock itself. Although the importance of UR occurrence in CR development is an issue that is still in debate (Mackintosh, 1974), the absence of an unconditioned response to the US provides a possible explanation for the fact that conditioning did not occur. Even though accelerative responding to the light with shock was of a significantly smaller magnitude than to the light alone, a depression in responding was also seen to the L- during pre-exposure trials and conditioning trials before shock onset. It therefore could have been due to different levels of responding of the subjects because of sampling error. A deceleration to shock is also not consistent with reports in the literature of large accelerations to shocks (Fitzgerald & Martin, 1976; Tighe et al., 1968). Moreover, there was no difference between the response to the tone alone and the tone with shock or responses to the shock alone and the shock with the light or the tone. Although pilot work preceding this study indicated that this shock sequence supported the development of classically conditioned cardiac responses, casual visual observations did not include any indication that rats found the US aversive (e.g., squealing, avoidance responses).

Summary

The prodedure used in Experimnt 1 was not successful in establishing stable conditioned heart rate responses using 28-sec lights and tones paired with shock. The absence of obvious URs is consistent with this outcome. This study was successful in demonstrating stimulus-specific spontaneous cardiac responses to the CSs used. It should be noted that the light and tone were not only of different modalities but that the tone CS was of a continuous nature while the light CS was intermittent. Differential responding may have been due to this factor. The experimental design was not successful in manipulating spontaneous responses by presenting CSs of one modality alone or combined during a session with a CS of another modality.

Experiment 2

Since the design used in Experiment 1 was not successful in establishing consistent conditioned heart rate responses, several aspects of the experimental design were in question as to their effectiveness. The primary event of interest was the US.

The method of applying shock to the superficial muscles is not a commonly used procedure in the literature. According to Barr (1978), the number of pain receptors in the superficial muscles is much less than that found in the skin. Since the skin is the location of shock stimulation used in studies finding consistent CRs and URs of large magnitudes (e.g., Fitzgerald & Martin, 1976; Teyler, 1970), it was decided that perhaps the US of Experiment 1 was responsible for the failure to establish consistent CRs. This idea was also supported by the absence of obvious URs to the shock sequence, although as mentioned previously, it has not been conclusively shown that UR occurrence is a determinant of CR development (Mackintosh, 1974).

It is also possible that a CS-US interval of 20 sec is not optimal for the development of conditioned responses in unrestrained rats. However, because of the interest in longer latency response components, the ISI for the second experiment remained at 20 sec.

Therefore, in Experiment 2, CSs were paired or unpaired with a shock US delivered to the chest through electrodes attached to the skin. Based on pilot work, a shock intensity of 2.0 mA was chosen because it consistently produced a large accelerative UR.

Since there was no effect of single vs combined stimulus modalities in the first experiment, this variable was not manipulated in Experiment 2. Two groups of animals were presented with light and tone CSs during the same session and these CSs were paired or explicitly unpaired with the US during conditioning trials in a typical discrimination paradigm.

Method

Subjects

The subjects were nine adult male albino rats, 190-240 days old, purchased with the rats from Experiment 1. Their mean weight at the time of surgery was 505 g, SD = 53 g. These rats were housed and maintained under the same conditions as those in Experiment 1.

Surgical Preparation

Two days prior to the start of testing, animals were anesthetized in the same manner as described previously and two heart rate monitoring electrodes were attached to the skin under antiseptic conditions. Each electrode consisted of a 3 mm solderless terminal clamped onto stainless steel suture wire that was looped 6-7 times through a 1 cm square section of skin. The electrodes were placed in the same positions as those in Experiment 1 but were not totally subcutaneous.

An animal saddle was fitted to each rat as an anchor for the electrode leads so as to avoid direct tension on the solderless terminals.

Apparatus

Animals were tested in the same apparatus as in Experiment 1 except that two small alligator clips were attached to each paracutaneous electrode for connection to the heart rate monitor.

The US was a series of three .5 sec 2.0 mA shocks provided by the Grason-Stadler shock generator and delivered through the ECG electrodes to the skin of the rats.

Procedure

Rats were randomly assigned to two groups, L+/T- and T+/L-, with 4 and 5 rats per group, respectively. These two groups underwent the same experimental paradigm as did the combined modality groups from Experiment 1, i.e., 20 stimulus pre-exposure trials, 60 conditioning trials and 20 extinction trials of both the light and the tone. Stimulus presentation order and intertrial intervals were those in Appendix Tables A1 and A2. Animals were tested in consecutive squads (details are listed in Appendix Table A4).

Results

Data from Experiment 2 were analysed exactly as the data from the combined modality groups of Experiment 1. The comparisons of interest were between responding to the light and the tone, between responding to paired and unpaired CSs and between responding over trials as evidence of habituation, acquisition or extinction.

Stimulus Pre-exposure

The mean heart rate change scores during the first 20 sec of L and T presentations of stimulus pre-exposure sessions are graphed in Figure 14 averaged over +/- and TBs. The expected differences between L and T are not obvious in this graph, but modality-specific responding was seen more clearly when the responses were averaged over +/- and trials per day of stimulus pre-exposure as graphed in Figure 15a (Day 1) and b (Day 2). The response to the light was biphasic and the response to the tone triphasic, as described in Experiment 1, but only during the second day (Panel b).

However, when the data were analysed, a significant main effect for CPs (*F*[19,91] = 21.46) and a significant interaction for L+/T+ x CPs (*F*[13,91] = 2.6) were revealed, neither of which provides evidence for modality-specific responding to the light and tone. The interaction appeared to be due to a greater magnitude of responding to both stimuli by the L+/T- group than by the T+/L- group during the last portion of the CSs.

There were no differences between responding to CS(+) and CS(-) that existed during pre-exposure. Although the same stimuli were used as in Experiment 1, the light and tone did not evoke modality-specific responding consistently throughout

Figure 14. The mean responses to the tone and light during stimulus pre-exposure averaged over +/- and TBs variables.

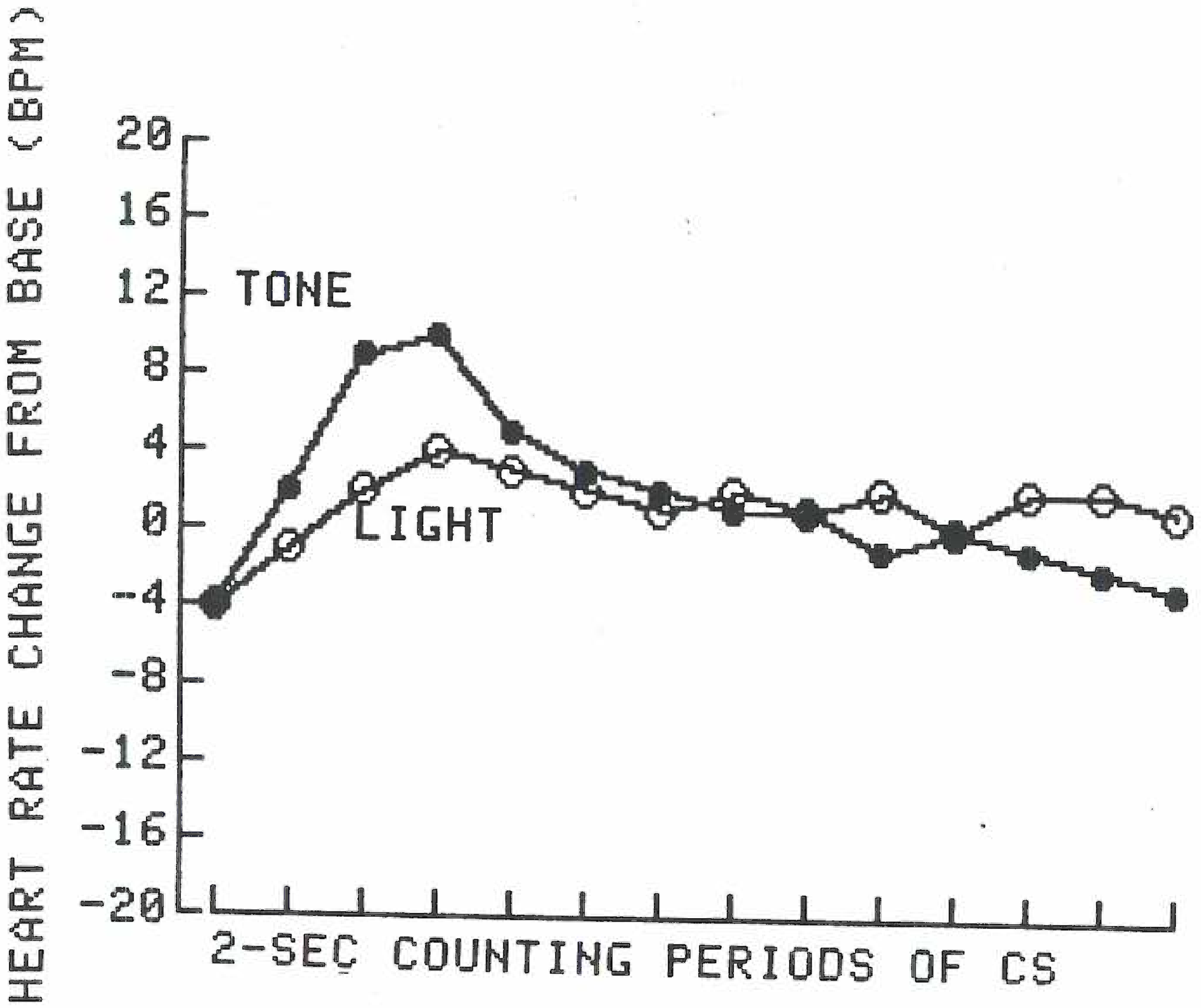
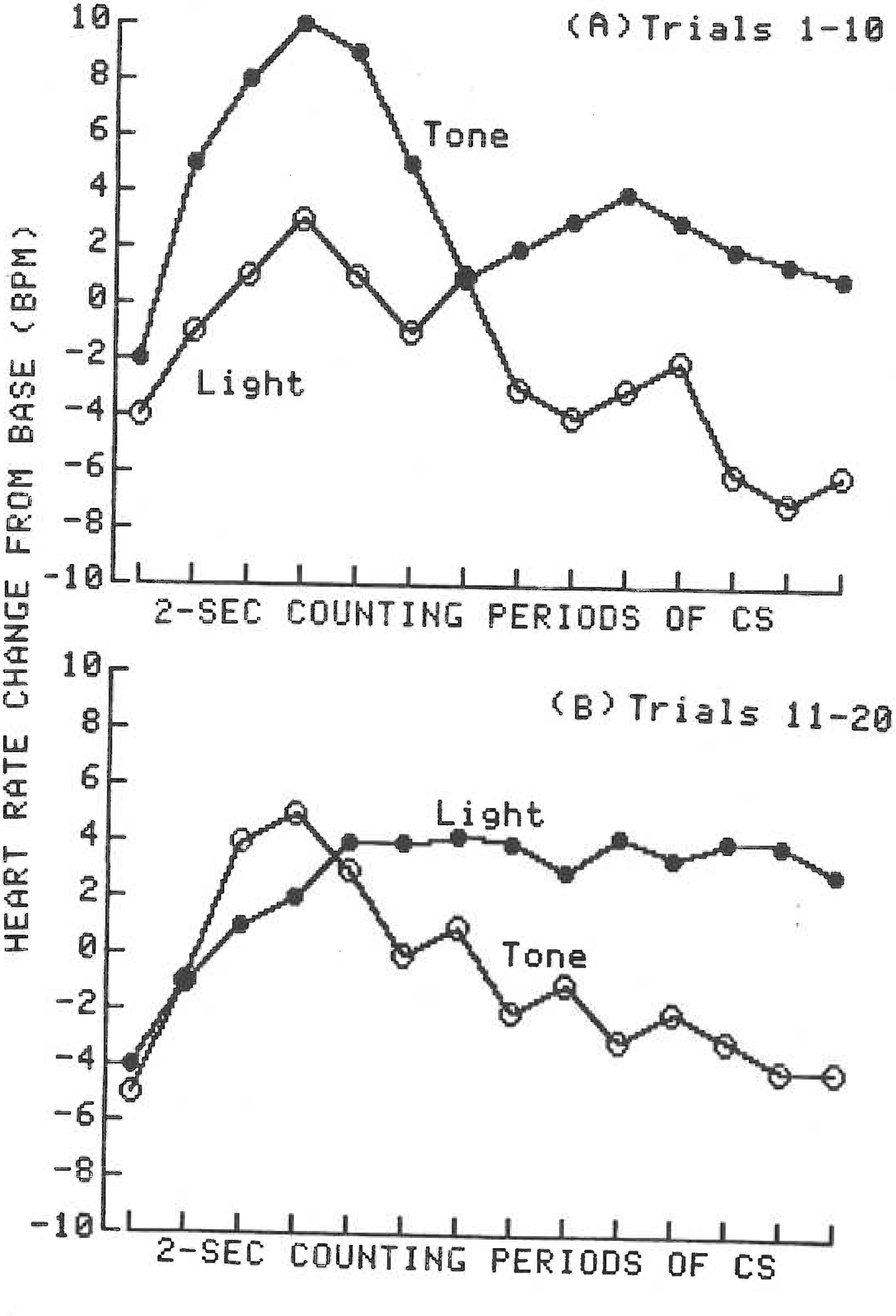

HEART RATE CHANGE FROM BASE (BPM)
20
16
12
8
4
0
-4
-8
-12
-16
-20
TONE
LIGHT
2-SEC COUNTING PERIODS OF CS

Figure 15. The mean responses to the light and tone during (a) trials 1-10 and (b) trials 11-20 of stimulus pre-exposure averaged over +/-.


HEART RATE CHANGE FROM BASE (BPM)
(A) Trials 1-10
Tone
Light
10
8
6
4
2
0
-2
-4
-6
-8
-10
2-SEC COUNTING PERIODS OF CS
(B) Trials 11-20
Light
Tone
10
8
6
4
2
0
-2
-4
-6
-8
-10
2-SEC COUNTING PERIODS OF CS

stimulus pre-exposure.

Conditioning

Figure 16 shows the mean HR changes to the L+, L-, T+ and T- averaged over all conditioning trials. Generally the responses to the CS+ were decelerative, reaching a maximum magnitude of 12-16 bpm. The responses to the CS- consisted of an initial small deceleration during the first CP which was followed by a gradual increase to a maximum acceleration of 8-12 bpm occurring during the fourth CP. The accelerative response to CS- then decreased in magnitude toward the end of the CS-US interval. These general observations were supported statistically by a main effect for +/- (*F*[1,7] = 13.63), a main effect for CPs (*F*[9,63] = 5.99) and a +/- x CPs interaction (*F*[9,63] = 3.90). There was no evidence of characteristic responding to the light or tone and no effect of TBs. The absence of a TBs effect but the presence of large differences in responding not seen during stimulus pre-exposure suggest that the changes occurred during the first few trials of conditioning.

In order to analyze more closely the development of the CRs during conditioning trials, a four-way ANOVA was run on the data from the first conditioning session. The between and within group variables were the same as the other ANOVAs used except that 2-trial blocks were analyzed instead of 10-trial blocks.

The mean responses to the CS+ and CS- are graphed in

Figure 16. The mean responses to the reinforced (+) and unreinforced (−) lights and tones during conditioning averaged over TBs.

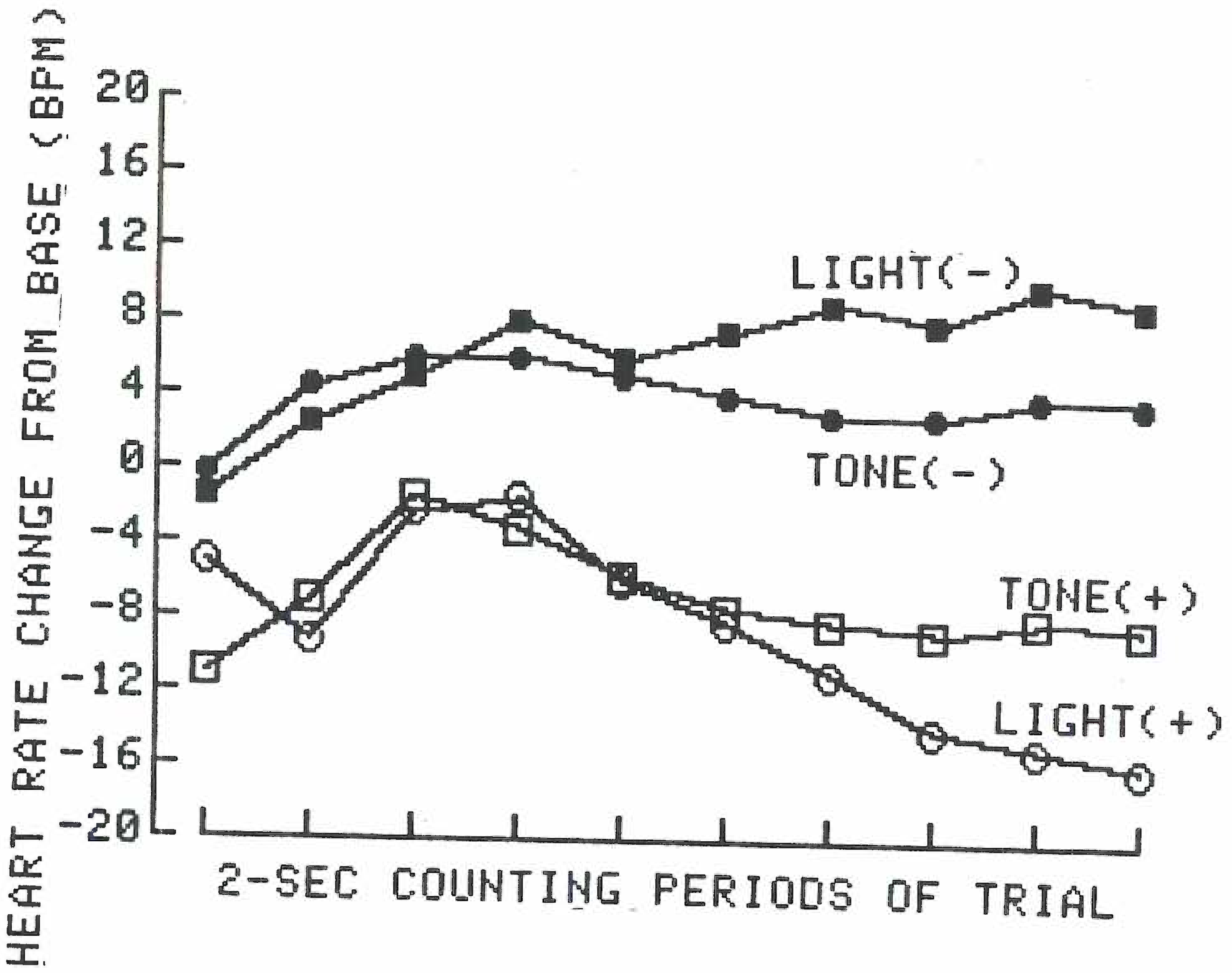

HEART RATE CHANGE FROM BASE (BPM)
20
16
12
8
4
0
-4
-8
-12
-16
-20
2-SEC COUNTING PERIODS OF TRIAL
LIGHT(-)
TONE(-)
TONE(+)
LIGHT(+)

Figure 17 averaged over L/T and CPs. The response magnitudes are similar during the first TB but diverge as conditioning continues. The response to the CS+ becomes generally more deceleratory and the response to the CS- generally more acceleratory across TBs. The ANOVA revealed main effects for +/- (*F*[1,7] = 7.00) and CPs (*F*[9,63] = 5.66) and a significant interaction for +/- x TBs (*F*[4,28] = 3.58). The interaction supports the observations made from Figure 17. It appears as if distinctive differences in the directions of the responses to CS+ and CS- were present by the third and fourth trials of conditioning. Although not merited by the ANOVA, the responses to the reinforced and non-reinforced light and tone are shown over both CPs and 2-TBs in Figure 18. It appears as if there is a change from responding resembling the spontaneous responses to the CSs during the first TB to responding determined by reinforcement schedule during the subsequent TBs. These changes were not statistically supported.

Extinction

The responses to the previously reinforced and non-reinforced light and tone are graphed in Figure 19 averaged over TBs. The response to the CS(+)s were large decelerations similar to those seen during conditioning trials as were the accelerative responses to the CS(-)s. There appeared to be no differences between responding to the L(+) and the T(+) or the L(-) and the T(-). The analysis on the

Figure 17. The mean heart rate responses to the CS+ and the CS- during 2-trial blocks of the first conditioning session. Responses are averaged over T/L and CPs variables.

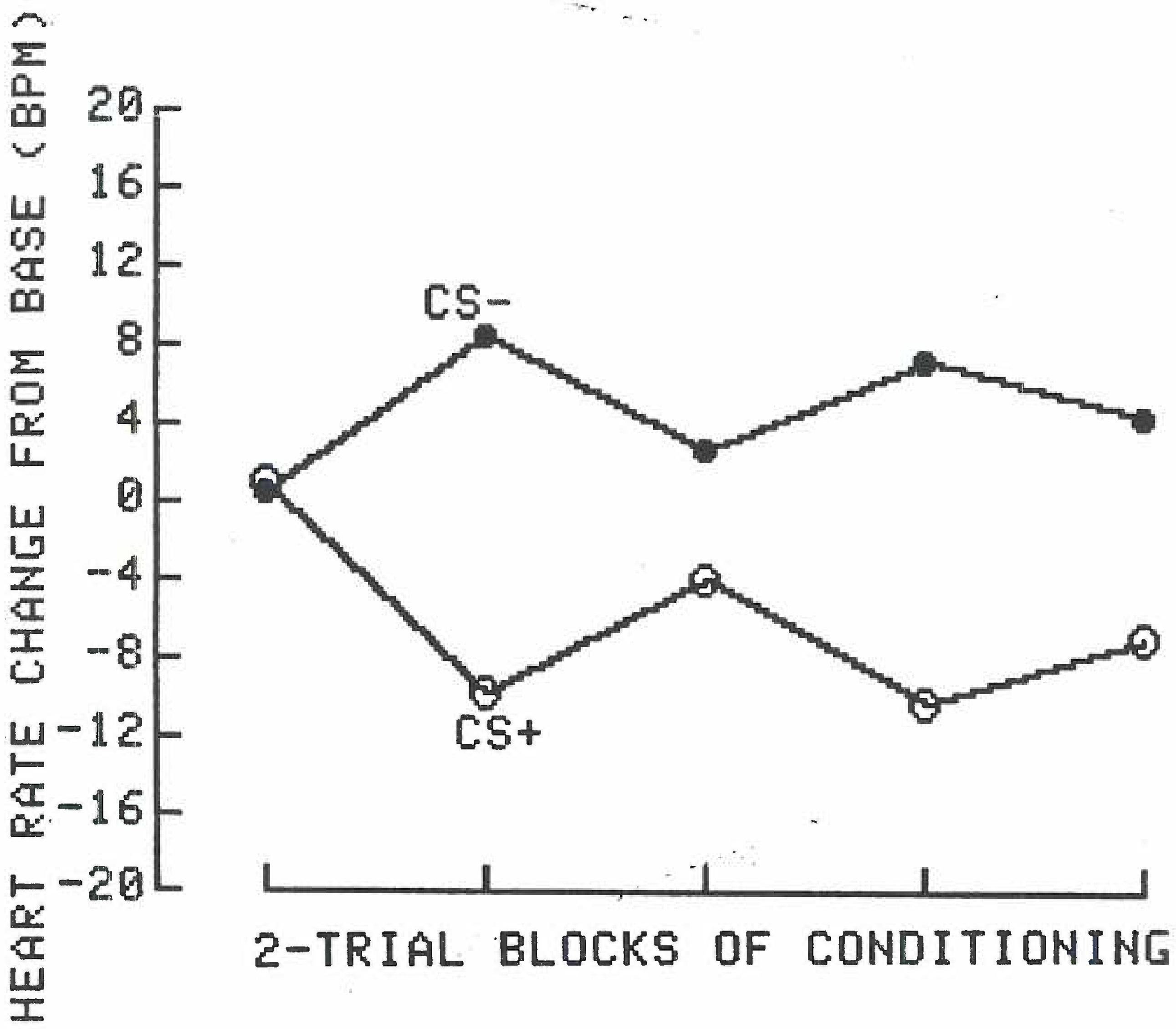

HEART RATE CHANGE FROM BASE (BPM)
20
16
12
8
4
0
-4
-8
-12
-16
-20
CS-
CS+
2-TRIAL BLOCKS OF CONDITIONING

Figure 18. The mean heart rate responses to the reinforced and nonreinforced light and tone over CPs during 2-trial blocks of conditioning (panels 1-5) on Day 1.

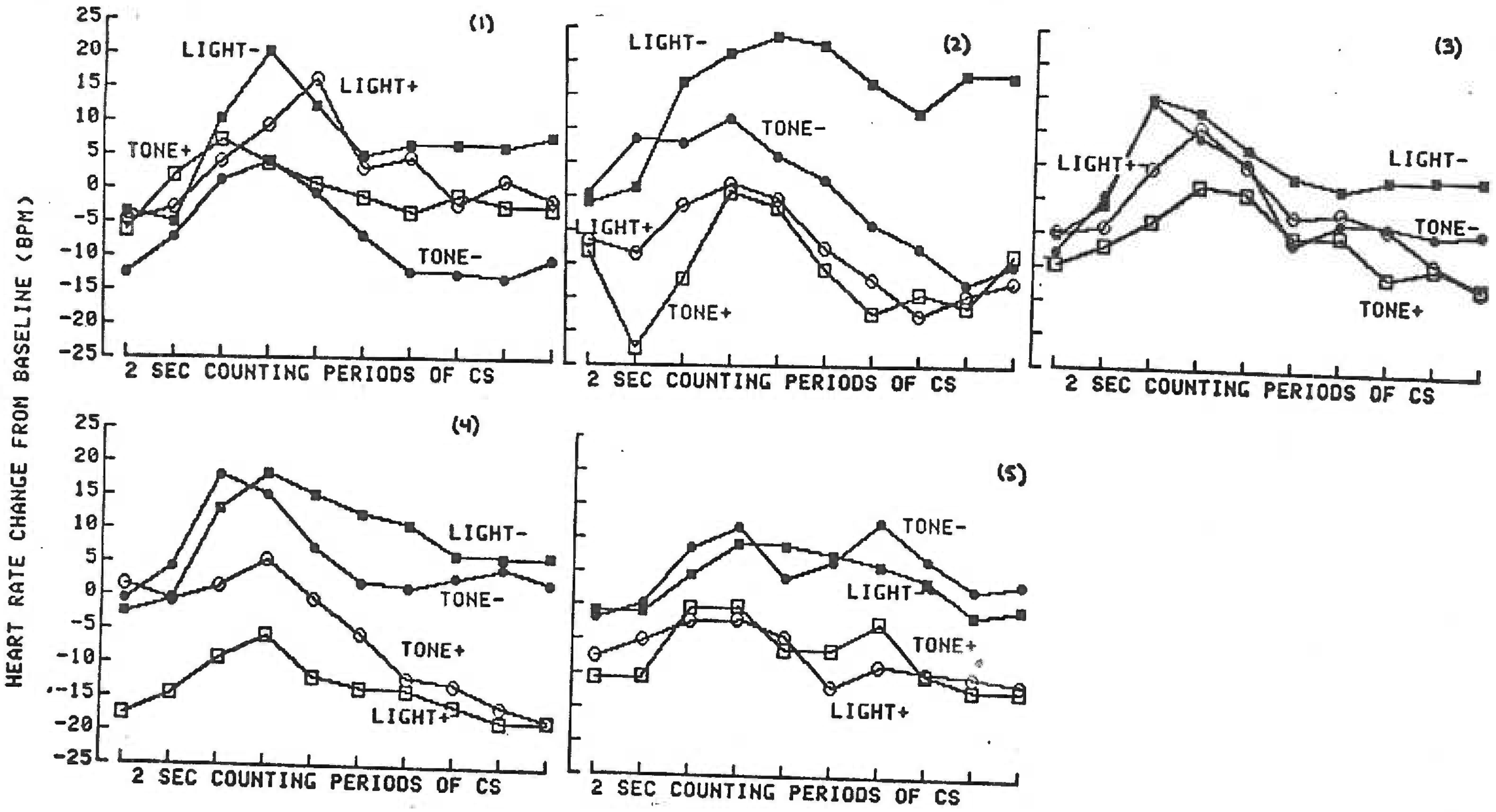
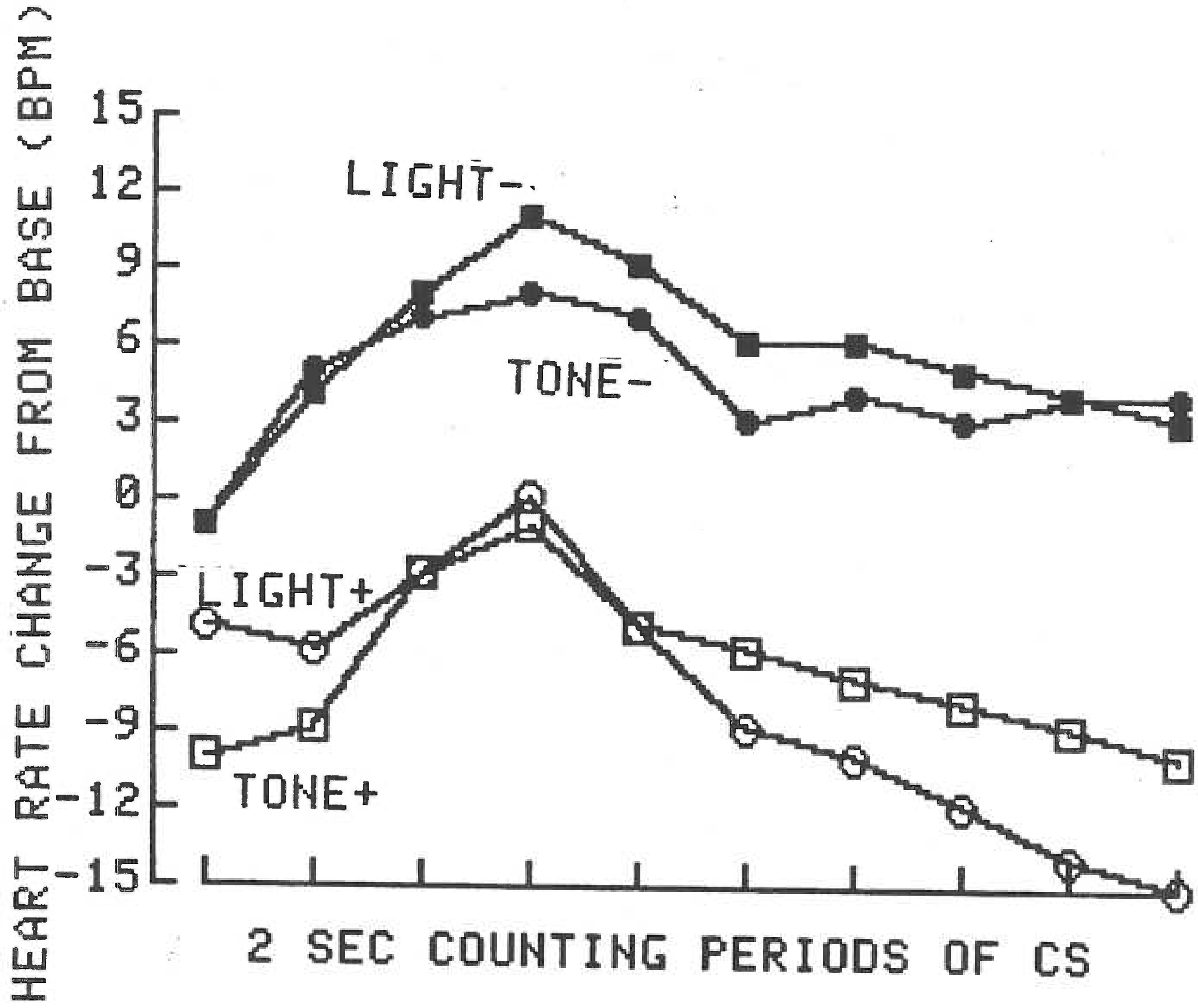

HEART RATE CHANGE FROM BASELINE (BPM)
25
20
15
10
5
0
-5
-10
-15
-20
-25
(1)
LIGHT-
LIGHT+
TONE+
TONE-
2 SEC COUNTING PERIODS OF CS
(2)
LIGHT-
TONE-
LIGHT+
TONE+
2 SEC COUNTING PERIODS OF CS
(3)
LIGHT+
LIGHT-
TONE-
TONE+
2 SEC COUNTING PERIODS OF CS
(4)
LIGHT-
TONE-
TONE+
LIGHT+
2 SEC COUNTING PERIODS OF CS
(5)
TONE-
LIGHT-
TONE+
LIGHT+
2 SEC COUNTING PERIODS OF CS

Figure 19. The mean responses to previously reinforced (+) and nonreinforced (−) lights and tones during extinction averaged over TBs.


HEART RATE CHANGE FROM BASE (BPM)
15
12
9
6
3
0
-3
-6
-9
-12
-15
LIGHT-
TONE-
LIGHT+
TONE+
2 SEC COUNTING PERIODS OF CS

data from extinction trials revealed significant effects for +/- (_F_[1,7] = 12.13), +/- x CPs (_F_[9,63] = 8.21) and L+/T+ x +/- x TBs x CPs (_F_[27,169] = 2.42). Separate three-way ANOVAs on the two groups showed a +/- x TBs x CPs interaction (_F_[27,98] = 1.87) for Group T+/L- and no effects of TBs for the other group. Both L+/T- and T+/L- groups exhibited +/- x CPs interactions (_F_[9,63] = 5.44 and 4.72, respectively). Two-way within-group ANOVAs on responses to the L and T presented to the T+/L- group revealed a TBs x CPs interaction (_F_[27,108] = 1.6) for the response to L(-) and only a significant CPs effect on the response to T(+). The TBs x CPs interaction for the L(-) stimulus is graphed in Figure 20 and appeared to involve an increase in the magnitude of accelerations during the second and fourth TBs (the second 5-trial block per extinction session). This is not consistent with what is usually expected of response changes associated with extinction (i.e., a decrement in magnitude of changes as non-reinforced presentations increase). It was therefore concluded that extinction of conditioned responses did not occur during the 20 non-reinforced trials per stimulus presented during the last portion of testing.

Unconditioned Responses

There is a striking difference between the responses during the last 22 sec of stimuli with shock and of stimuli alone as shown in Figure 21 when the responses were averaged across TBs of conditioning. The response to CS plus shock was

Figure 20. The mean responses to L- of Group T+/L- over 5-trial blocks of extinction (TB1-4).

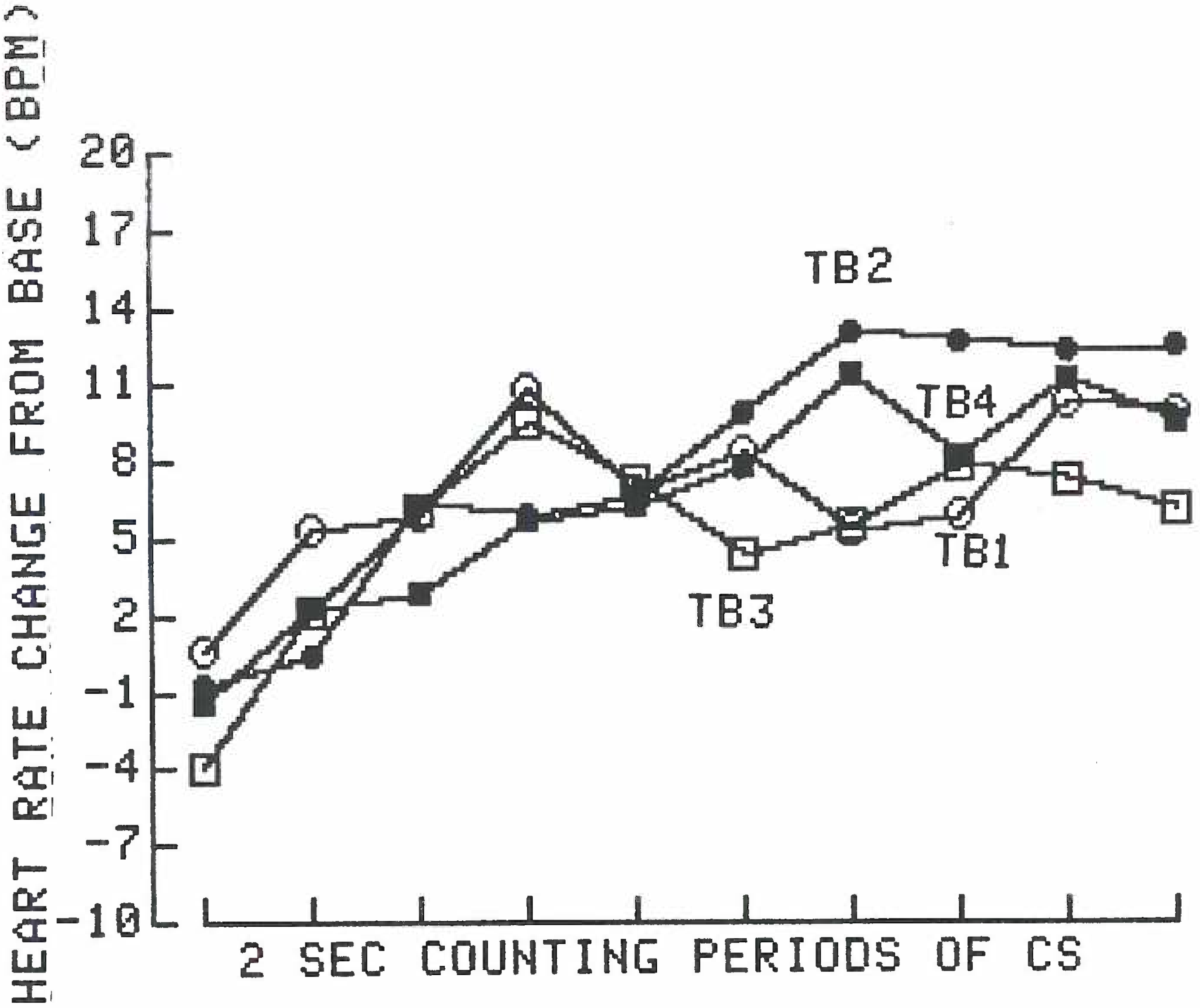
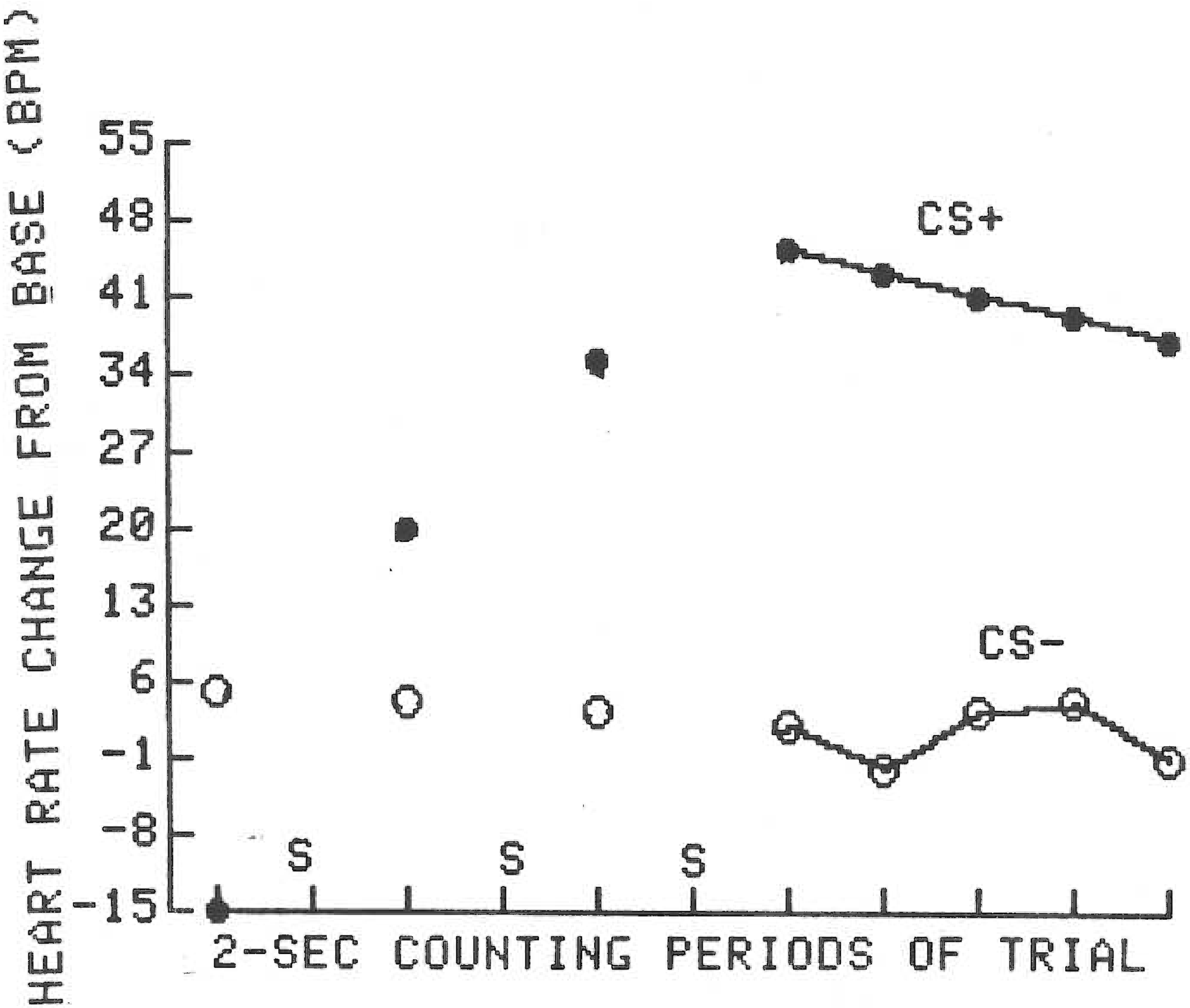

HEART RATE CHANGE FROM BASE (BPM)
20
17
14
11
8
5
2
-1
-4
-7
-10
2 SEC COUNTING PERIODS OF CS
TB2
TB4
TB1
TB3

Figure 21. The responses to the CSs plus shock (S = 2-sec counting period during which shock occurred for the reinforced stimulus only) as compared to those to the CSs alone during the last 22 sec of conditioning trials.


HEART RATE CHANGE FROM BASE (BPM)
55
48
41
34
27
20
13
6
-1
-8
-15
2-SEC COUNTING PERIODS OF TRIAL
S
S
S
CS+
CS-

a large acceleration of about 40 bpm as compared to almost baseline responding to the non-reinforced stimuli. Responding changed from a deceleration before the first shock pulse to an acceleration that increased in magnitude across the measurement period. There was a significant +/- effect (F[1,7] = 8.58), CPs effect (F[7,49] = 22.03) and +/- x CPs interaction (F[7,49] = 18.48).

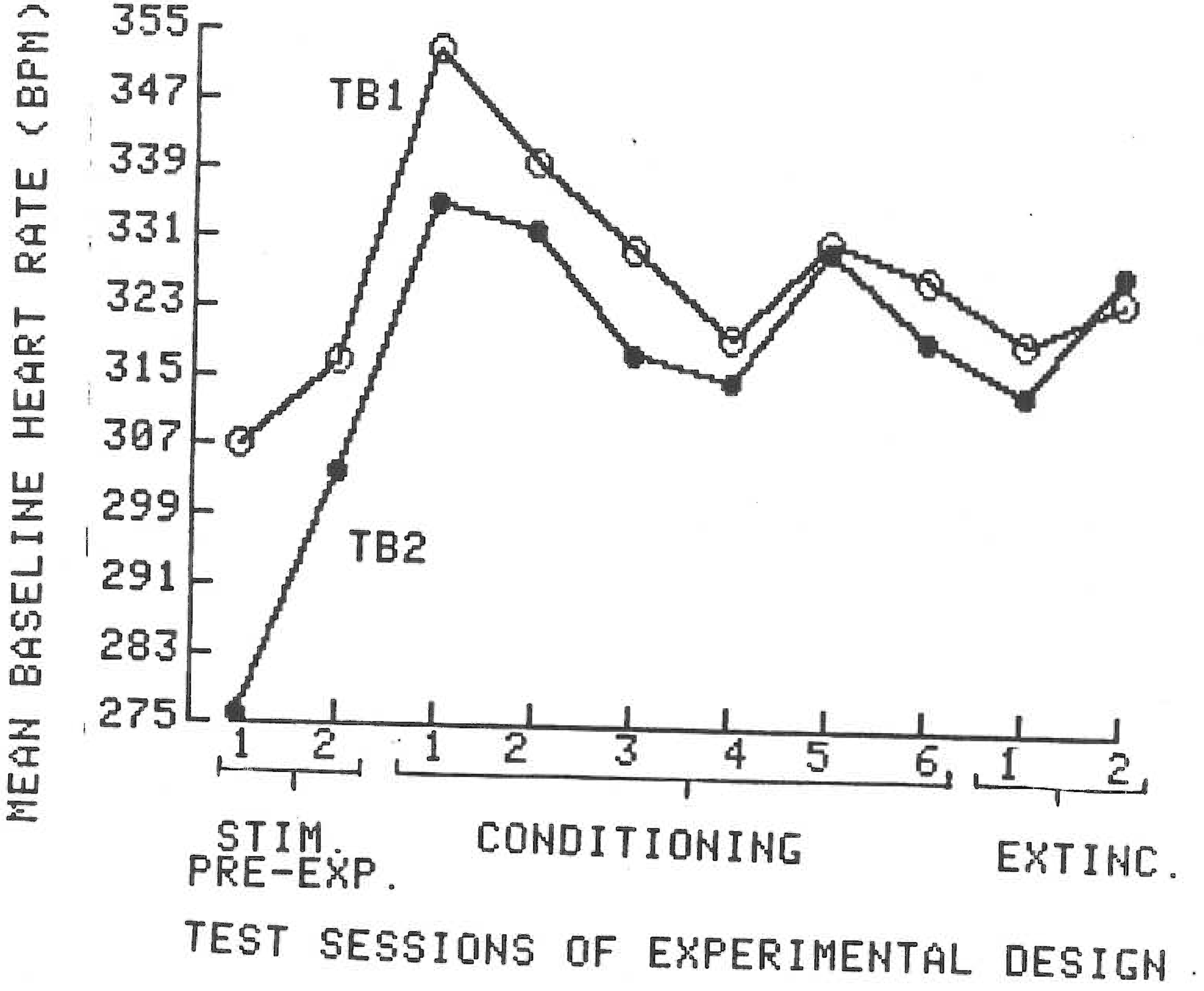
There was no difference in responding between the L and T when the shock was present and no difference when the shock was not present. It was concluded that the cutaneous US was able to elicit an accelerative UR that did not diminish in magnitude for at least 10 sec after the last shock pulse was administered.

Baseline Heart Rate

Mean baseline heart rates measured prior to each CS type are shown in Figure 22 averaged across stimulus types (there were no apparent differences between stimuli or groups). Baseline heart rates appeared greater during the first as compared to the second TB of each session and the difference remained fairly constant across sessions. Baselines increased on the first day of conditioning (when shock was introduced) and then generally decreased as testing progressed.

A four-way ANOVA using groups (L+/T+) as the between groups variable and +/-, days and TBs per day as within group variables revealed a days effect (F[9,63] = 4.93) and a TBs effect (F[1,7] = 7.49). Thus, there were no differences in

Figure 22. Mean baseline levels of responding during the first (TB1) and second (TB2) trial blocks of each test session averaged over groups.


MEAN BASELINE HEART RATE (BPM)
355
347
339
331
323
315
307
299
291
283
275
TB1
TB2
1
2
1
2
3
4
5
6
1
2
STIM.
PRE-EXP.
CONDITIONING
EXTINC.
TEST SESSIONS OF EXPERIMENTAL DESIGN

baseline levels of heart rate that existed prior to the start of CS+ and CS- trials that might have accounted for the differences in responding evoked by these events.

Discussion

Differential spontaneous cardiac responses to light and to tone CSs appeared to be present only during the last 10 trials of stimulus pre-exposure but were not found to be significantly different when compared over all pre-exposure trials. The responses during the second day were similar in topography to the responses described in Experiment 1 (i.e., triphasic response topography to the tone CS and biphasic topography to the light CS). Responding to the light CS appeared to be diminished during the first day of CS exposure which may have led to the nonsignificant difference between tone and light responses. This effect may have been due to the age of the animals, previous handling and the amount of time spent in a different environment as compared to rats from the first experiment.

The large difference between responses to CS+ and CS- led to the conclusion that the experimental design, especially the 2.0 mA shock to the skin, was able to support the development of conditioned discriminatory responses. Decelerations of about 20 bpm were observed during the CS+ and accelerations of about 20 bpm during the CS-, regardless of the stimulus modality of the events. This is contrary to what was proposed by Wilson (1969) who would have expected conditioned responses that resembled the spontaneous responses to the stimuli but would be of a greater magnitude after the CSs were paired with the US.

These data do not replicate the findings of acceleratory responses to CS+ and deceleratory responses to CS- by Martin and Fitzgerald (1980) in unrestrained rats, however the shock US was not comparable (i.e., a 20 Hz train of 0.1 msec, 240-V, DC pulses delivered to the tail). They partly replicate findings by Teyler (1970) who found deceleratory responses to the CS+ but not accelerations to the CS-, although he had expected both types to occur. Teyler used shock delivered to the skin by the same method used in Experiment 2. The results of the second experiment also correspond with findings in other species, both restrained and unrestrained (Notterman et al., 1952; Sutterer et al., 1972) of deceleratory CRs and acceleratory ORs.

The differences due to conditioning were still quite obvious during extinction trials and did not appear to decline in strength even after 20 nonreinforced exposures to each stimulus. The response to the unconditioned stimulus was a large acceleration of about 40 bpm, which is consistent with URs cited in the literature.

GENERAL DISCUSSION

It appears as if spontaneous cardiac responses occurred, as expected by Wilson (1969), that were specific to the stimulus presentations and habituated slowly, if at all. The absence of response magnitude depression even after 100 exposures during Experiment 1, however, was not expected and implies a more permanent type of response. These spontaneous responses were not affected by the presence of other stimulus modalities during testing or by low intensity shock delivered to the superficial muscles of the chest either during the CSs or during the intertrial intervals. Modality-specific responding was abolished when a high intensity US delivered to the skin was paired or unpaired with the stimulus events, and was replaced by large magnitude CRs that were deceleratory during the CS+ and acceleratory during the CS-. The change from modality-specific, spontaneous responding to modality-independent, conditioned responding occurred within the third and fourth TBs of the first conditioning session.

The second experiment, in which long duration interstimulus intervals were used, was successful in establishing conditioning. The long ISI allowed maximal observation during both experiments of response topographies, including tri- and bi-phasic components.

It appears from the second experiment that when a stimulus was paired with an aversive US, a CR resulted that was in a direction opposite (deceleratory) that of the UR (acceleratory). This coincides with Schneiderman's hypothesis of compensatory or preparatory CRs, but the design of this experiment does not manipulate the US in such a way so as to support such an hypothesis directly. Fitzgerald and Hoffman

(1976) measured the CRs in groups receiving USs that either elicited a decelerative UR (ammonia fumes) or an accelerative UR (electric shock), and saw no effect on direction of the CRs paired with these USs. Although Schneiderman might deny that these USs were actually eliciting responses to which the compensations would be of opposite direction, those data cast considerable doubt on his hypothesis. The data from Experiment 2 also do not support views that the CR is determined solely by the original response to the CS as proposed by Dykman (1967).

The proposal that characteristics of the CR are determined by both the OR and the UR is neither supported nor totally disputed by the results of these experiments. Although the CRs of the second study did not resemble in any way the spontaneous responses to the light or tone CSs, and were not affected by the different CS modalities, it is possible that such response components were obscured by other components determined by the UR. More specifically, since the URs were of such large magnitude relative to the spontaneous responses, CR components determined by the former may also be considerably larger than those determined by the latter, thereby obscuring any response components attributable to modality-specific responding.

It would be of interest in future experimentation to demonstrate such codetermination of the conditioned response by manipulating both the spontaneous responses and the unconditioned responses to the CS and US, respectively. If an interaction between such manipulations is found then it would support a multiple component theory of conditioned response development.

SUMMARY AND CONCLUSIONS

Two experiments were conducted that observed the effects of different CSs (an intermittent light and a continuous tone) on both unconditioned and classically conditioned heart rate responses in an attempt to delineate the relationship between these two types of responses. In both experiments, heart rate was monitored in unrestrained rats before, during and after a classical Pavlovian conditioning procedure.

In the first experiment, an attempt was made to manipulate the spontaneous response to the light and tone by presenting one or two stimuli of different modalities during stimulus pre-exposure sessions. The CSs were then paired or explicitly unpaired with a shock sequence delivered subcutaneously in an attempt to establish classically conditioned responses. Non-reinforced CSs were presented after conditioning sessions so as to measure extinction effects.

Stimulus-specific heart rate responses were observed in unrestrained rats to the tone and light stimuli that did not habituate after 100 trials and were not affected by the number of stimulus modalities per session. Responses both to the light and the tone began with an initial deceleration which changed to an acceleration. Responding to the tone returned to baseline levels before the end of the 28-sec stimulus but responding to the light remained accelerated throughout the CS. These responses were not affected by differential reinforcement with the subcutaneous US, therefore it was concluded that classical conditioning did not occur.

The second experiment attempted to isolate the reason for failure of conditioning in Experiment 1. A different location (the skin) was designated for the site of US delivery, known to contain more pain receptors. This shock was paired and unpaired with the light and tone CSs of Experiment 1 in a typical discrimination paradigm. Discriminative CRs developed within the first four conditioning trials that were were deceleratory to CS+ and acceleratory to CS-. These conditioned responses were independent of the CS modality and did not exhibit extinction after 20 non-reinforced trials per stimulus presented after conditioning.

It is of further interest to determine the proportion of response components determined by the US and what portion (if any) are determined by the CS in future experimentation. It is proposed that by maniplulating both the spontaneous response and the unconditioned response such a determination could be made.

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Table A1. Three Quasi-random Lists of 21 Intertrial Interval lengths (in sec).

List 1	*List 2*	*List 3*
120	150	240
240	180	150
270	240	90
210	90	210
280	180	120
90	120	180
250	210	270
210	270	90
90	90	120
180	270	270
150	180	210
120	240	240
240	210	150
270	120	180
150	150	210
240	270	150
270	120	240
90	90	120
120	240	270
180	150	90
210	210	180

Table A2: Four Lists of Stimulus Presentation Order

List A	List B	List C	List D
1	2	1	2
2	1	1	2
1	2	2	1
1	2	2	1
2	1	2	1
2	1	1	2
2	1	1	2
1	2	1	2
1	2	2	1
1	2	2	1
2	1	1	2
2	1	2	1
1	2	2	1
1	2	1	2
2	1	1	2
2	1	2	1
2	1	2	1
1	2	2	1
1	2	1	2
2	1	1	2

NOTE: 1 = CS+

2 = CS-

Table A3: Schedule of Group Testing Order (partially randomized).

Number	Group
1	L+
2	L-
3	T+
4	T-
5	L+/T-
6	T+/L-

Squad Number	Group Number of Subject in Each Squad Start Time: A.M.-------------------→P.M.
1	1, 2, 6, 4, -, -, -, -
2	3, 6, 5, 2, -, -, -, -
3	6, 5, 4, 2, 1, 3, -, -
4	4, 3, 5, 6, 2, 1, -, -
5	2, 1, 4, 5, 1, 4, 3, 6
6	1, 3, 2, 6, 4, 5, -, -
7	3, 5, 5, 3, -, -, -, -
8	6, 4, 1, 3, 5, 2, 2, -
9	5, 2, 3, 1, 2, 6, 4, -

Table A4: A Schedule of Group Running Order for Experiment 2 (partially randomized).

Number	Group
1	L+/T-
2	T+/L-

Squad	Group Numbver of Subjects in Each Squad Start Time: A.M.-----------------P.M.
1	1, 2, 2, 1, 1, 2
2	2, 2, 1, -, -, -