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The University of Oklahoma, Ph.D., 1974
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HEART RATE OF LIZARDS OF THE FAMILIES IGUANIDAE AND AGAMIDAE
AS AN INDICATOR OF BEHAVIORAL RESPONSE TO SOCIAL STIMULI

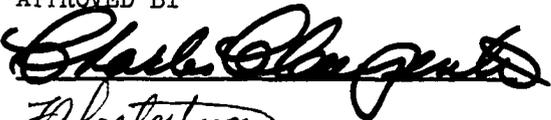
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degree of
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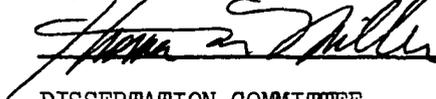
HEART RATE OF LIZARDS OF THE FAMILIES IGUANIDAE AND AGAMIDAE
AS AN INDICATOR OF BEHAVIORAL RESPONSE TO SOCIAL STIMULI

APPROVED BY









DISSERTATION COMMITTEE

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HEART RATE OF LIZARDS OF THE FAMILIES IGUANIDAE AND AGAMIDAE
AS AN INDICATOR OF BEHAVIORAL RESPONSE TO SOCIAL STIMULI

CHAPTER I

INTRODUCTION

Many animals exhibit elaborations of motor patterns, vocalizations, or a combination of these resulting from the process of ritualization, in which a motor pattern evolves into a communicative act. Often emphasis of morphological structures and color patterns emphasizes motor patterns, making them stand out from the background of environmental noise (Blest, 1961; Cullen, 1966; Lorenz, 1966). These ritualized motor patterns are sometimes phylogenetically released from their original function, and operate solely in a new context of communication (Tinbergen, 1952).

Ritualized displays have been described in many vertebrates and invertebrates. In recent years the lizard families Iguanidae and Agamidae have received considerable attention (Bussjaeger, 1967, 1971; Carpenter, 1966, 1967; Carpenter and Grubitz, 1961; Clarke, 1965; Ferguson, 1969; Jenssen, 1969; McCardell, 1970; McKinney, 1971). The lizards studied have a species-specific aggressive display characterized by head-nodding or push-up type movement to a particular temporal cadence. This has been called the display-action-pattern (DAP) by Carpenter (1967).

Many of these lizards also conspicuously display color patterns. Posturing of the male Merriam's canyon lizard (Sceloporus merriami; Iguanidae) involves body compression so that a keel is formed along the longitudinal axis of the mid-ventral abdomen (Carpenter, 1961), increasing the visibility of striking ventral colors, particularly during the combative breeding season.

Several species also have elaborate structures to emphasize distinctive color patterns. In the iguanid genera Norops (Kastle, 1963) and Anolis (Carpenter, 1965; Jenssen, 1969; Ruibal, 1967; Greenberg and Noble, 1944), a large and often brightly colored dewlap pumps in and out during the display, increasing the information content (Rand and Williams, 1970) and making the signal more conspicuous. Among the agamids, the inflated and darkened dewlap of the bearded dragon (Amphibolurus barbatus) resembles a black beard (Carpenter et al., 1970). The frilled lizard (Chlamydosaurus kingii) erects a large neck frill, or shield, during aggressive encounters (Schmidt and Inger, 1957).

A long-standing problem related to behavior ritualization concerns sign-stimuli reception. How an animal distinguishes a meaningful signal from the mosaic of environmental noise is still unclear. A real understanding of the mechanisms underlying animal behavior requires both ethological and physiological considerations (Konishi, 1971). One way to achieve this understanding is to examine changes in physiological parameters associated with social behavior.

I have examined several questions concerning interrelationships between heart rate and ritualized aggressive behavior in lizards.

- 1) During stress of a social origin, are there detectable changes

in the heart rate of a lizard?

2) How does the heart rate in a subordinate lizard change as he observes the display of a dominant conspecific?

3) Are there differences in basal heart rate that can be correlated with an animal's social rank?

4) Does heart rate change when a lizard is presented with a visual stimulus conveying information important to its survival?

5) One expects an interrelationship between sensory input and cardiac phenomena, but how predictive and acute is it?

6) What is the relative importance of the bob and the various components of the male's color pattern in terms of aggressive communication?

If the effect on heart rate via the sensory somatic circuit is distinct enough, heart-rate changes could be used to distinguish those portions of an animal's display that actually function in aggressive communication.

Jenssen (1969) found that female Anolis nebulosus orient toward a film-loop of a normal male conspecific display more readily than to an altered one, indicating stimulus discrimination. Heart rate and electrocardiographic waveform changes may be more refined response indicators than orientation. Another indicator of response to stimuli is the electroencephalogram (Hunsaker and Lansing, 1962), but it has not been investigated extensively.

Heart-rate responses of lizards to social stimuli have been given little study. This problem requires an understanding of the manner in which heart-rate parameters change as a response to change in stimuli and stimulus levels. The effect of gross levels of excitement or non-

specific stressors on heart rate and ECG waveforms must be observed to determine both patterns and parameters of response to these stimuli. The display-action-pattern of a lizard is such a subtle stimulus. Carpenter and Sassaman (1968) have developed techniques for contiguous recording of ECG and display cadence. Temporal relationships between the dominant's display and the ECG of both animals are easily observed.

The eastern collared lizard (Crotaphytus collaris collaris; Iguani-
dae) is ideal for observing various components of the ritualized display using heart-rate changes as an indication of the communicative function of the display components. The aggressive display is characterized by hopping movements while posturing with arched back and extension of a small gular pouch or dewlap (Mosley, 1963; Greenberg, 1945). The males are brightly colored: turquoise body speckled with yellow, a bright yellow-orange head, and a black double-collar. By presenting laboratory lizards with latex rubber models representing collared lizards in aggressive posture, one can emphasize various components of their display while monitoring physiological parameters.

CHAPTER II

LITERATURE REVIEW

Heart-rate change has been used in a variety of behavioral studies. Thompson and co-workers (1968a, 1968b) used it to measure the importance of various sounds to starlings (Sturnus vulgaris). Playback of starling distress calls caused heart-rate elevation.

Social psychologists (Kagan and Lewis, 1965) used changes in heart rate as measures of attentiveness in infants tested at six and at thirteen months of age. Cardiac deceleration occurred as a response to attention to visual and auditory stimuli in adults (Lacey, 1959; Lacey et al., 1963) and infants (Kagan and Rossman, 1964). Heart-rate changes in infants were used as a measure of stimulus discrimination on a visual cliff by Campos et al. (1970); prelocomotory human infants were able to discriminate visual cliff depth, responding to the deep side of the cliff by cardiac deceleration.

Miller and DiCara (1967) demonstrated shaping of heart-rate change via instrumental conditioning, even with skeletal muscular action blocked with curare. Their results stress the importance of the central nervous system versus skeletal muscle action mediating cardiac changes.

Hunsaker and Lansing (1962) found a longer electroencephalographic response in Sceloporus olivaceous to models carved and painted in a non-aggressive posture.

Reptilian Heart Function

Vertebrate heart function has a variety of interrelated controlling mechanisms including hormone action, tissue oxygen demand and neural control (White, 1968b). Vertebrates have direct cardiac innervation by the autonomic nervous system (Johansen and Martin, 1965; White, 1968a). Although White (1968b) found no evidence of sympathetic cardiac innervation in reptiles, both Harris (1963) and Khalil and Malek (1952a, 1952b) indicate sympathetic nerves to the heart at least in the Agamidae.

Most studies of reptilian electrocardiography use either the conventional Einthoven (1903) lead system and terminology (Buchanan, 1909) or some modification thereof. A common electrode configuration involves placing electrodes on the limbs (Licht, 1965; Francis and Brooks, 1970) or in the trunk area near the heart (Mullen, 1967; Akers and Damm, 1963), usually with a modified Einthoven triangular lead system. The Einthoven system was developed for humans, but does not maximize the signal strength of electrocardiographic waveforms in lizards. With few exceptions, electrocardiograms recorded from lizards are similar to those of humans recorded with standard electrode placement (Bayley, 1958).

Temperature is an important factor affecting heart rate in reptiles. Akers and Damm (1963) studied its effect on heart rate and ECG waveforms in the freshwater turtles Chrysemys (Pseudemys) elegans and Graptemys geographica. They found a slower and more variable heart rate at lower temperatures than at higher ones. As the temperature increased from 10° to 40° C, the amplitude of the QRS complex and T-wave both decreased but the amplitude of the P-wave showed slight decline at temperatures above 30° C. No change occurred in the duration of the S-T and the P-R intervals, but the duration of the QRS complex as a percentage of the

R-R interval increased, reflecting a decrease in the R-R interval or an increase in the overall heart rate. Licht (1965) used the difference between resting and maximal heart rate as an indicator of potential for cardiac increase in four species of lizards at different temperatures. The differences between resting and maximal heart rates were greatest at the preferred body temperatures of each of the species studies. Johansen (1959) found changes of five to six beats $C^{-1} \cdot \text{min}^{-1}$ in three species of Norwegian snakes and lizards at temperatures ranging from 8° to 30° C. He also recorded changes in the ECG waveforms, notably a longer P-wave, Q-T interval, P-R interval, and QRS complex at lower temperatures. Direction of temperature change is extremely critical (H. F. Landreth, Jr., personal communication), since at the same temperature heart rate is lower during warming than cooling. This difference is probably due to lag between core and cloacal temperatures.

The sympathetic and parasympathetic autonomic connections to the heart are reported to have a seasonally differing effect (Khalil and Malek, 1952a). In Uromastyx aegyptia the inhibitory effects of the parasympathetic nerves are more pronounced during winter months; conversely, the accelerating effects of the cardiac sympathetic nerves are more marked in the summer.

Heart rate depends on many physiological and environmental variables including temperature, season, age, size, time of day, acclimation temperature, nutritional status, and prior experience (Mullen, 1967). Using heart-rate changes rather than absolute heart rate as the dependent variable serves partially to correct for these when looking for behavioral effects.

CHAPTER III

MATERIALS AND METHODS

The use of ECG's to elucidate behavior required development of techniques differing from those usually used in other studies. With unanesthetized and unrestrained lizards, electrodes had to be firmly implanted and leads shielded to prevent noise caused by movement of the wires and by electrical interference.

Electrode Design

Three types of electrodes were used, depending on the study. Recordings from Amphibolurus barbatus were made using Grass platinum needle electrodes with a five-foot plastic insulated lead; the very small gauge of the subcutaneous electrodes caused minimum trauma to the animals, only two of which were available for study. The large size of these lizards facilitated securing the electrodes with masking tape around the tail.

Some recordings, notably those from Agama agama, were made using the tips of 22-gauge stainless steel hypodermic needles soldered to a several-inch length of fine, single-stranded, plastic-coated copper wire. The three electrodes were connected to a miniature transistor socket embedded in a balsawood saddle glued to the back of the animal. Although this method worked well as a long-term implant with Agama, other lizards, especially Crotaphytus collaris, died after a few days. Difficulty was

also encountered in fastening the balsawood saddle to the backs of lizards having small granular scalation as in Crotaphytus.

A third electrode was a small one-inch brass-coated steel safety pin soldered to two-conductor, stranded and shielded phonograph cart-ridge pick-up wire. One safety pin was soldered to each of the two conductors; a third pin, a ground electrode, was soldered to the shield. Except for the shaft, the entire pin and distal 5 mm of wire were coated with red glyptal insulating varnish. The proximal end of the wire was soldered to the end of a stranded, shielded, and insulated audio cable; the shield of the cable was connected to the ground electrode and grounded.

Electrode Placement

Many lizard families have hearts with spherical ventricles in which much of the QRS depolarization complex is canceled before reaching the body surface (Abildskov and Klein, 1962; Mullen, 1967). A method of subcutaneous electrode placement was employed producing an excellent QRS complex, and in some species, notably Agama agama and Sauromalus obesus, a very clear and complete electrocardiogram (ECG) including the QRS complex, and both the T- and P-waves. A strong QRS complex is especially necessary in differentiating heart beats from the interference of muscle potentials in active animals.

Actual placement of the electrodes on the animals varied, but the usual configuration was a bi-polar electrode system (Bayley, 1958). In the dorsal-ventral (d.v.) system used, the positive exploring electrode was inserted either on the left dorsal side of the lizard immediately anterior to the pectoral girdle, or along the left side of the neck.

The negative reference electrode was located ventrally, just to the right of the midline and at the posterior apex of the sternum. A ground electrode was placed on the back just anterior to the right pelvic region. This electrode positioning was done empirically to give a strong positively deflected QRS complex as the depolarization or accession wave moved across the ventricle toward the exploring or positive electrode (Mullen, 1967). Since the positive electrode was located dorsally and anteriorly, the positive deflection of the strong QRS complex indicated that the accession wave moves forward, facilitating expulsion of ventricular blood during ventricular systole.

Recording Instrumentation

Electrocardiograms were recorded with a Grass model 79 two-channel polygraph, using the Grass model 7P6 EKG preamplifier and the Grass model 7DAC driver amplifier. An oscilloscope was used to monitor the ECG signal. This insured, without switching on the oscillograph writer of the polygraph, that the electrodes were properly attached.

A thermistor (Yellow Springs Telethermometer) was inserted into the lower colon of the lizard, or placed to monitor the air temperature on the bottom surface of the cage. The temperature was periodically checked and noted on the chart during the ECG recordings (Figure 8).

CHAPTER IV

BEHAVIORAL PARAMETERS OF HEART-RATE CHANGE

Introduction

Several questions are of interest regarding changes in heart activity resulting from various types of physical and behavioral stresses.

1) In terms of gross stimulation induced by rough handling of lizards, what changes occur in heart rate, variability of heart rate (irregularity of instantaneous heart beat), and ECG waveforms and their temporal relationships?

2) How does heart rate change in response to potential predators or prey (e.g. small rodents)?

3) What is the degree of stimulus discrimination involved in cardiac changes? How specific must a stimulus be to evoke a cardiac response from an animal?

4) How does heart rate change in response to conspecifics?

Effect of Gross Physical Stimulation on

Cardiac Function

Although considerable information about heart-rate changes in lizards with respect to environmental variables exists, there have been no studies on the effect of gross non-specific physical stimuli on lizard heart rates. Knowledge of the time course of recovery from physical stimuli allow these to be controlled in later studies.

Heart-Rate Changes

When an animal is stressed, three factors can act to cause an immediate increase in heart rate. Direct sympathetic innervation of the heart causes cardioacceleration when the sympathetic system is stimulated, while the adrenal medulla is stimulated by sympathetic neurons under conditions of stress. Two cardioacceleratory actions are induced by the adrenal medullary catecholamines; epinephrin acts directly on the heart to increase the rate of contraction, norepinephrin acts indirectly by serving as a general vasoconstrictor, maintaining blood pressure under stress. Both hormones also act to effect an increase in calorigenesis, inducing an increased oxygen demand on the stressed animal. This increased oxygen demand in turn requires an increased cardiac output. In general, the effects of sympathetic stimulation on the circulatory system are the same as those occurring at the beginning of physical activity (Tepperman, 1962).

The most obvious cardiac change with gross stimuli is a change in heart rate. Recordings were taken from several species: Dipsosaurus dorsalis, Sauromalus obesus, Agama agama, and Agama atra; all obtained from commercial dealers.

Recording of Heart Rate -- ECG's were recorded using either Grass platinum needle electrodes or brass-coated safety-pin electrodes in subdermal d.v. configuration. Lizards were housed in a 15-gallon aquarium or 3 ft³ observation cage during recording trials.

Animals were left alone for at least a half hour after electrodes were positioned until a steady heart rate was obtained and they stopped moving about vigorously; resting or basal ECG's were then recorded for

several minutes. The lizards were removed from the enclosure and handled roughly to induce stress and obtain maximum excitement. Rough handling consisted of tapping the lizard on the head and causing it to bite and in some cases taking colonic temperatures. Immediately after this rough treatment, the animals were returned to their cage and ECG's recorded until a relatively constant heart rate was again evident or until they moved about causing secondary heart-rate elevation.

Analysis and Results -- Heart rates were analyzed for consecutive 30-second intervals: two intervals during the resting period immediately prior to the rough handling, and as many as sixteen intervals following this treatment. The number of post-treatment intervals varied due to complications resulting from movement by the lizard or other interference causing recording difficulties. Ten trials were conducted with two Sauromalus obesus, and two trials each with single specimens of Dipsosaurus dorsalis, Agama agama, and A. atra.

Several possible familial differences in cardiac response are evident (Figure 1). The initial post-stimulus heart-rate increment was greater ($p < .01$) for the iguanids ($237.1 \pm 9.86\%$ (mean \pm S. E.)) than for the agamids ($166.0 \pm 2.54\%$). The agamids showed little decline in heart-rate increment during the post-stimulus period. In the case of Sauromalus, the heart-rate increment was significantly ($p < .05$) below the immediate post-stimulus level 2.5 minutes following the beginning of the post-stimulus recording, while 3.5 minutes after stimulation the heart rate of A. agama was still 62% above the base rate.

The percentage decrease in Sauromalus heart rate appears to be exponential as a function of time. A semilogarithmic plot (Figure 2)

Fig. 1. Decline in heart rate in four species of lizards following initial heart-rate elevation due to rough handling. The sample size for Sauromalus obesus was 7 to 10, and was 2 for the other species.

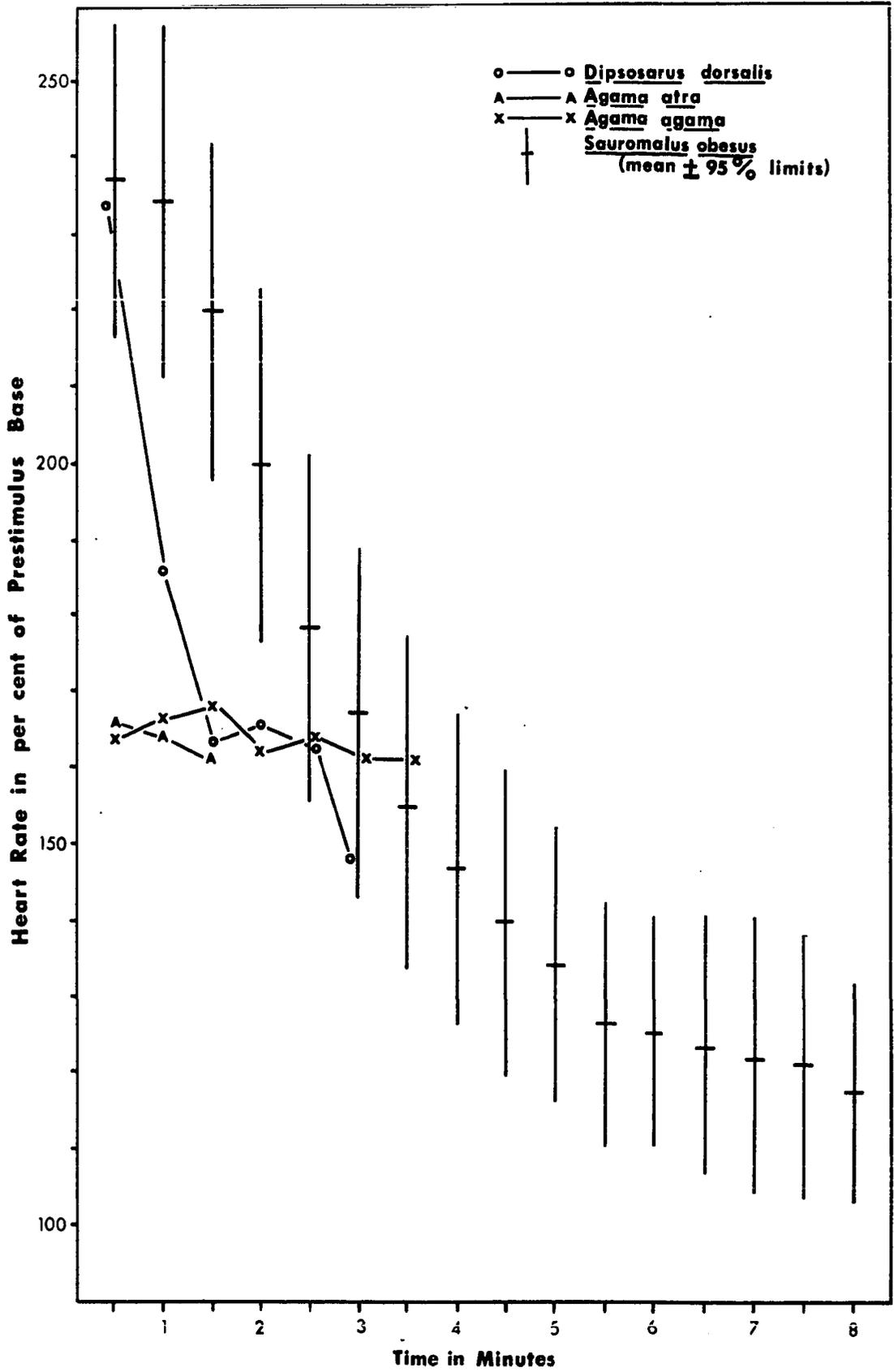
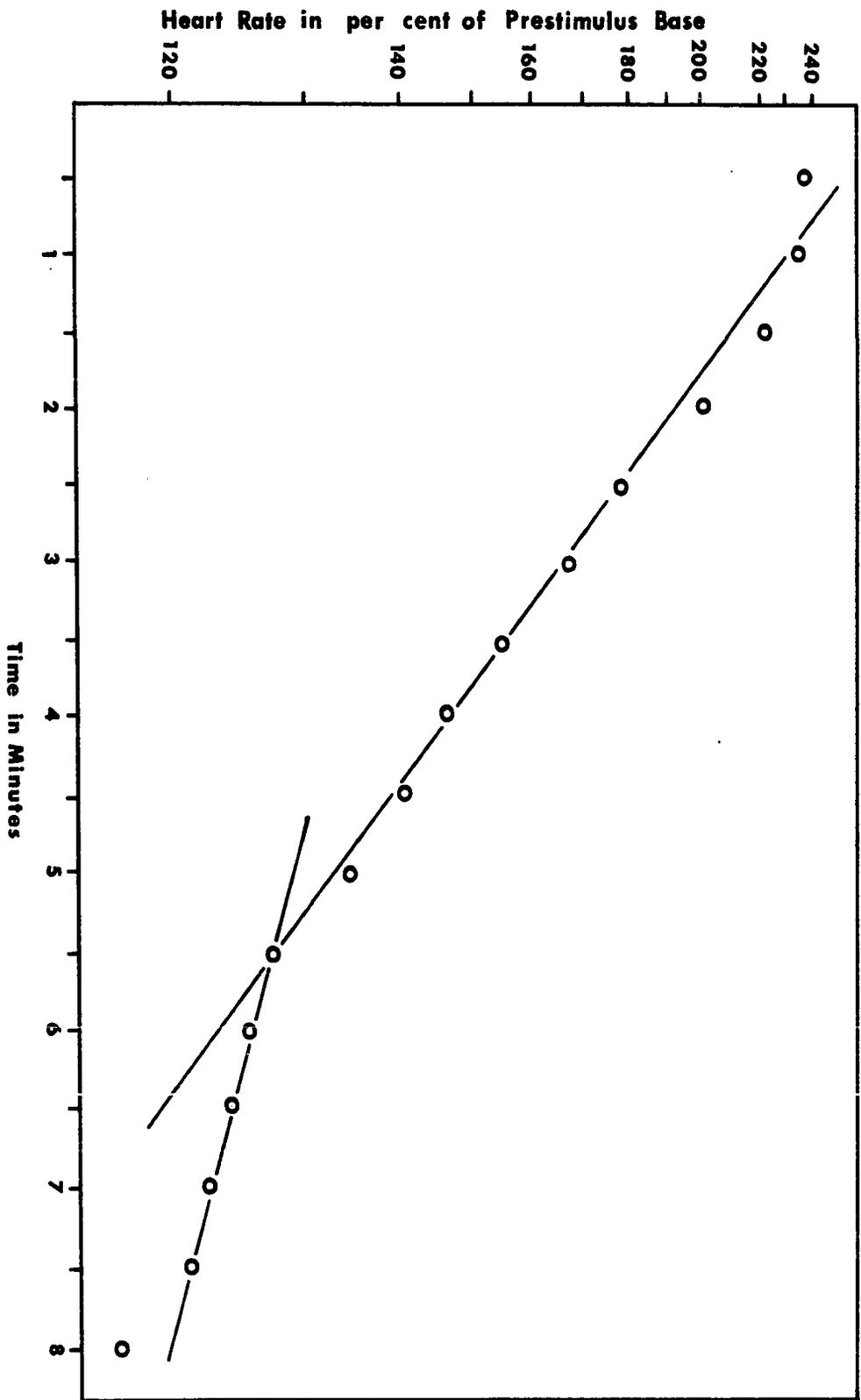


Fig. 2. Semi-log plot of heart rate following initial heart-rate elevation due to rough handling for Sauromalus obesus, using the data from figure 1.



suggests such a decline. Similar responses have been observed in water snakes (Natrix: William Gehrman; personal communication).

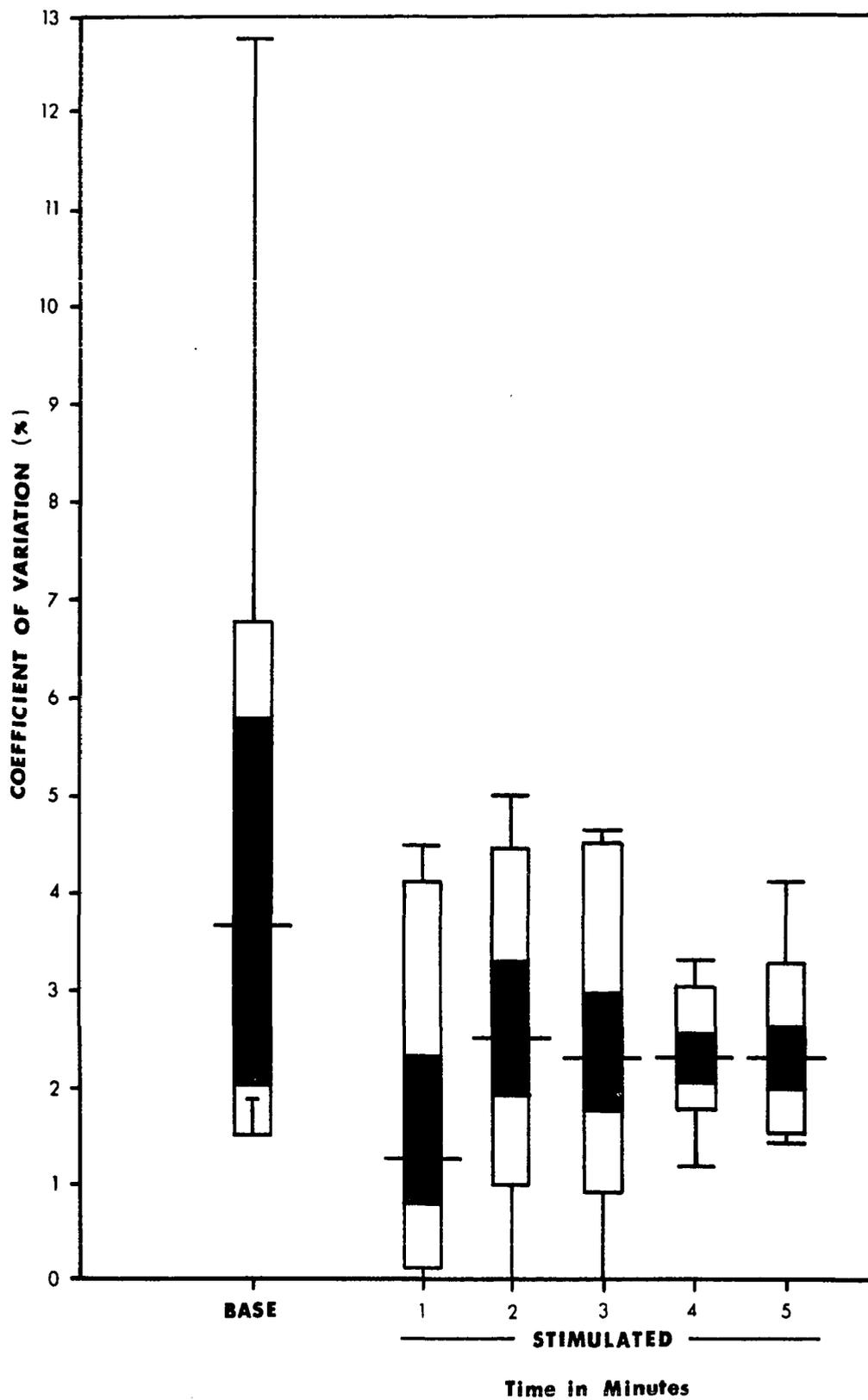
The exponentially decreasing curve of heart-rate increment with time in Sauromalus is possibly related to the rate of metabolic breakdown of catecholamines released during stimulation. There may be two separate physiological events involved in the return of heart rate to its base level: a rapid decline for the first five minutes following stimuli, and a subsequent more shallow decrease in rate. The exact nature and significance of these two events are not understood.

Heart-Rate Variability

Variability of instantaneous heart rates for successive intervals following stimuli were compared with each other and with the pre-stimulus variability in Sauromalus for the same trials reported above. Changes in heart-rate variability during cardioacceleration may be indicative of changes in the underlying physiological mechanisms causing the increase in heart rate.

To measure variability, coefficients of variation (C.V.'s) were determined for samples of 25 consecutive heart beats, consisting of 25 beats immediately preceding the stimulus and the initial 25 beats in each one-minute interval following stimulation for a maximum of five minutes. Coefficient of variation is used as a measure of variability since it corrects for differences in variance resulting from differing sample means (Sokol and Rholf, 1969). The C.V.'s were transformed to degrees from arcsin tables (Rholf and Sokol, 1969) and the mean and its statistics of dispersion were calculated, and reconverted back to C.V.'s in per cent (Figure 3).

Fig. 3. Coefficients of variation for base heart rate and five one-minute intervals following gross physical stimulation in Sauromalus obesus. The black bar represents the 95% confidence limits of the mean, the open bar represents the standard deviation of the mean, the vertical line represents the range, and the horizontal line represents the mean. (Sample size=ten for the base and minutes 1 and 2, and nine for minutes 3, 4, and 5.)



Although there was a tendency for the lizards to display a lower mean variability after stimulation, this trend was significant ($p < .05$) only during the first minute (Figure 3). After stress the animals generally remained stationary in their cage, showing no overt movement. Most of the evoked cardioacceleration was probably due to an increase in sympathetic tone. Cardioacceleration resulting from hormone influences may induce a steadying effect upon the instantaneous heart rate. If so, one would expect the time-course of the post-stimulus C.V.'s of figure 3 to be roughly exponential and reciprocal to the heart-rate decline curve of figure 1, which it is. As will be noted later (Figure 13) cardioacceleration in a displaying dominant animal resulted in a significantly greater variance of the instantaneous heart rate, suggesting that cardioacceleration resulting from increased muscular activity induces an erratic heart beat.

Changes in the ECG Waveforms

A third quantitative measure of ECG change in response to gross levels of stimulation is found in the temporal relationships of various components of the ECG and in changes in shape and polarity of its component waveforms. As heart rate increases, concomitant decrease in the T-P interval (ventricular repolarization to atrial depolarization) representing diastole would be expected. Shortening of other time units, such as the R-P interval and the R-T interval, indicates possible changes in the electrical characteristics of the myocardium. The applicability of subtle changes in the temporal and spatial patterns of the ECG cycle of lizards to quantify response to stress situations is apparant, but whether changes in the ECG pattern would occur with enough resolution to enable

their quantification with subtle stimuli of a behavioral nature, even with more sensitive electrode locations, is questionable.

Temporal Changes -- Waveforms were compared for resting and excited lizards for which QRS-, T-, and P-waves were clearly evident. The intervals between the various waves were measured for 20 consecutive heart beats immediately preceding and following rough handling stress. In table 1, the mean length of the intervals between waveforms are compared for each of the primary cardiac cycle intervals for Sauromalus obesus (two observations) and Agama agama (one observation) at rest and after stress.

The expected decrease in the diastolic time was represented by decreases in the T-P interval and in the T-P interval as a percentage of the total length of the cardiac cycle. There were concomitant decreases in the duration of the R-T and the P-R intervals, even though they made up a larger percentage of the cardiac cycle after excitement than before. The decrease in the duration of the R-T and P-R intervals together represent a decrease in systolic time.

The waveform intervals in this study differed from those found by Mullen (1967) in that the R-T (Q-T) intervals of resting lizards were all consistently lower than than his (60-63% of total). Even excited lizards were consistently below this level by a small amount. P-R intervals for resting Agama and for excited Agama and Sauromalus were in line with the range of 25 to 30% reported by Mullen. The differences may be attributable to temperature or other environmental variables.

Changes in Waveform -- The same essential elements of the human ECG cycle are found in that of the lizard, but during the resting non-excited

Table 1. ECG waveform changes in resting and excited lizards, expressed in mm distance between waveforms for ECG's recorded at $10 \text{ mm} \cdot \text{sec}^{-1}$. Each mean and its S.E. are derived from 20 consecutive beats. The per cent of the total R-R interval for each interval is given in parentheses. The differences between the resting and the excited means are significant ($p < .001$) in all cases.

<u>Individual Animal</u>	<u>Interval</u>	<u>Resting</u>	<u>Excited</u>
<u>Agama agama</u>	R-R	20.35 + .089 (100.00)	14.98 + .025 (100.00)
	R-T	10.03 + .025 (49.26)	7.98 + .025 (53.26)
	T-P	4.83 + .091 (23.71)	2.95 + .034 (19.70)
	P-R	5.50 + 0.0 (27.03)	4.05 + .034 (27.04)
<u>Sauromalus obesus</u>	R-R	29.95 + .798 (100.00)	12.15 + .300 (100.00)
	R-T	8.00 + 0.0 (26.71)	7.02 + .235 (57.80)
	T-P	17.03 + .798 (56.84)	2.06 + .036 (16.96)
	P-R	5.00 + 0.0 (16.69)	3.16 + .084 (26.02)
<u>Sauromalus obesus</u>	R-R	20.25 + .093 (100.00)	8.60 + .046 (100.00)
	R-T	7.88 + .050 (38.88)	4.50 + 0.0 (53.33)
	T-P	7.40 + .093 (36.54)	1.50 + 0.0 (17.44)
	P-R	4.93 + .041 (23.32)	2.60 + .046 (30.23)

state in a lizard the T-wave is negative in polarity with respect to the R-wave (R-wave upward, T-wave downward). In the human ECG this wave is positive with respect to the R-wave. Differences occur in the timing of the waveforms between human and lizard ECG's due to cardiac structural differences.

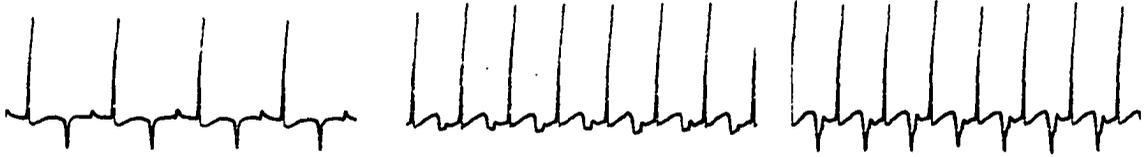
During excitement, the deflection of the T-wave often changes polarity. At times this occurs simultaneously with reduction of the T-P interval, so that both T- and P-waves summate with a resultant upward pen deflection of greater magnitude than represented by the subsequent R-wave. This upward deflection of the T-wave gradually disappears and becomes a downward deflection (Figure 4). No attempt was made to quantify these observations. The behavioral implications of this change in T-wave polarity are unclear. In humans an upright T-wave is indicative of an earlier repolarization of the left side of the ventricle, while an inverted T-wave is indicative of an earlier repolarization of the right portion of the ventricle (Hoff et al., 1939). It is possible that blood catecholamine changes during gross stress cause changes in blood flow rates to the ventricle, resulting in changes in the pattern of ventricular repolarization.

Response to Small Mammals -- A Behavioral Stimulus

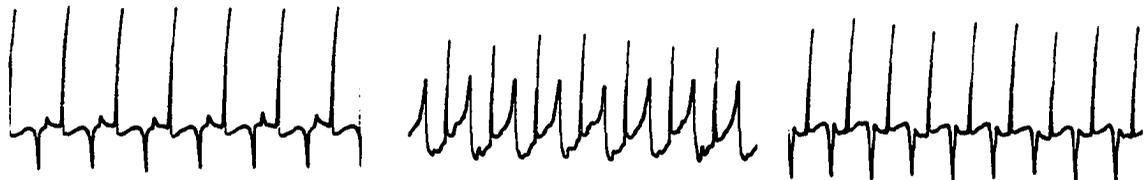
Behavioral parameters associated with agonistic communication are far more subtle than non-specific stressors, and may not lead to the same quantitative and qualitative responses. During the course of several observations, I noted that many lizards responded aggressively and vigorously to the presence of Mongolian gerbils (Meriones unguiculatus) and Merriam's kangaroo rats (Dipodomys merriami). The heart rate of liz-

Fig. 4. Electrocardiograms of two Agama agama and one Sauromalus obesus showing changes in the waveforms following gross physical stimulation. All of the recordings in each sequence were taken within a span of several minutes.

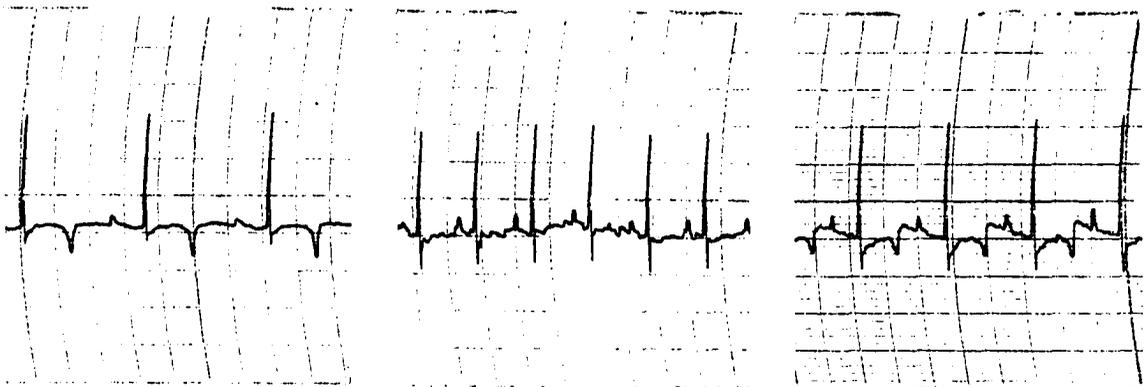
Agama agama



Agama agama



Sauromalus obesus



Resting

Stimulated

Resting

zards increased immediately after exposure.

Heart-rate changes in lizards resulting from exposure to small rodents involve behaviorally oriented responses related to the lizard's need to either chase down and consume prey, or to prepare to fight or flee from a potential predator. The major portion of the observed cardioacceleration is probably due to a general increase in autonomic and muscular tone rather than overt activity, since cardioacceleration is immediate and does not parallel the overt muscular activity of the animal.

Behavioral Responses

Similar behavioral responses to the presence of gerbils were noted in lizards from each of four families observed (Agamidae, Chameleonidae, Gekkonidae, and Iguanidae). The behaviors appeared aggressive in nature, but differed in intensity in different species. Amphibolurus barbatus demonstrated the least response to a gerbil with a display consisting of a slow extension and darkening of the gular "beard" and a general attentive posture. This low intensity response on the part of A. barbatus may be due to similarity of the gerbils to the mice that they were fed in the laboratory. However, a Chameleo sp. partially maintained on a diet of newborn mice had the strongest aggressive reaction to the presence of a gerbil. This response consisted of marked lateral compression of the body, and within several minutes, a change in color. Movement toward the mammal was accompanied by hissing sounds. In one instance, a chameleon, with a snout-vent length of less than 13 cm, attempted to reach a gerbil by leaping a 30 cm gap between two tables. Even after falling to the floor, the lizard still behaved aggressively toward the

gerbil. An Anolis equestris responded in a manner similar to the chameleon, changing color from green to dark brown within five minutes. Several Sauromalus obesus responded to a gerbil by aggressive attacks with sinuating movements of the torso interspersed with lunges at the intruder. A gerbil was used to elicit an aggressive posture in a male Pachydactylus bibroni after numerous attempts had failed to elicit an aggressive response to its conspecific.

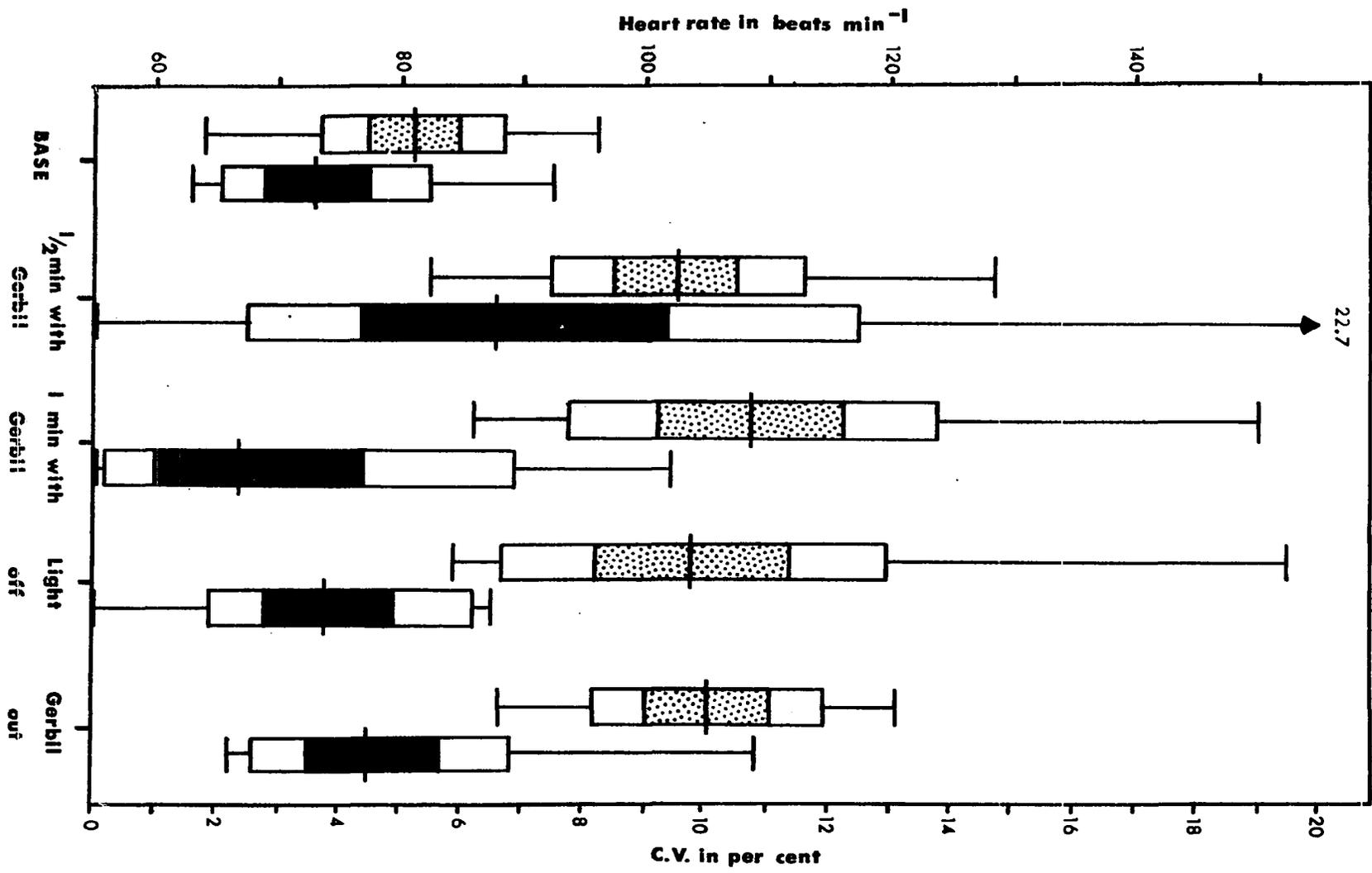
Cardiac Response

To quantify changes in heart rate and heart-rate variability with presentation of a small mammal, 18 trials were run with six Crotaphytus collaris. Heart rates were recorded using the d.v. electrode configuration. In these trials, a 30-second base-line was followed by two 30-second intervals during which the gerbil was presented to the lizard along with a light, one 30-second interval with the light off and the gerbil still in the cage, and a final 30-second interval after the gerbil was removed, but with the light still off (Figure 5).

Presentation of the gerbil resulted in a heart-rate increment maintained for as long as several minutes while the gerbil was present. This response did not decline immediately with the gerbil removed from sight (light turned off); indeed, in all of the intervals, the heart rate was above the base level ($p < .05$).

Variability of heart rate as measured by the coefficient of variation of 20 beats in each interval increased over the base level only during the first 30-second interval following introduction of the gerbil ($p < .05$). This increase in variability with the introduction of a gerbil is not what would be predicted from figure 3 based on physical stimuli, indicat-

Fig. 5. Heart rate in $\text{beats}\cdot\text{min}^{-1}$ and coefficient of variation of heart rate in per cent for Crotaphytus collaris in response to a gerbil in its cage (18 trials). The 30-second intervals sampled were contiguous. The coefficients of variation were converted to arcsines as discussed (page 16). Solid error bars indicate the C.V., stippled error bars indicate heart rate. Statistics of dispersion are as in figure 3.



ing the possibility of different physiological mechanisms causing cardio-acceleration in the initial 30 seconds of gerbil presentation. During the initial 20 heart beats following the introduction of the gerbil not only was the mean C.V. greater but also the standard error of the mean C.V. and the range of C.V.'s tended to be greater during this phase than any other, indicating not all of the trials showed a marked initial increase in variability of the instantaneous heart rate.

Looking at immediate changes in heart rate in C. collaris upon presentation of the gerbil, the instantaneous heart rates were compared for a total of 18 trials with six animals (Figure 6). The last five beats before stimulus and the first 20 measurable beats following the introduction of the gerbil were compared for all trials to obtain a mean curve of heart-rate change upon presentation of the gerbil. In several cases two to three seconds at the beginning of the presentation were obscured by muscular activity. Heart rate increased immediately with presentation of the gerbil, and within three heart beats was significantly ($p < .05$) greater than the base level. A plateau was reached within 12 to 13 beats.

Discrimination of Stimulus

Lizards respond to a variety of stimuli with changes in heart rate and other parameters of cardiac function. How can one differentiate between stimuli of differing biological importance to an animal on the basis of cardiac response? Figure 7 shows the instantaneous heart rate of a male Amphibolurus barbatus presented with several stimuli of presumably differing values of importance to the animal. Response to the introduction of a brick and of a Sauromalus obesus in the enclosure was immediate but short-lived cardioacceleration lasting about six heart

Fig. 6. Instantaneous heart rate of Crotaphytus collaris for five beats prior to, and twenty beats following, the introduction of a gerbil into the lizard's cage. The instantaneous heart rate for each beat is expressed as the mean and its 95% confidence limits for 18 trials (with six lizards) in beats·min⁻¹ and in the R-R interval in mm.

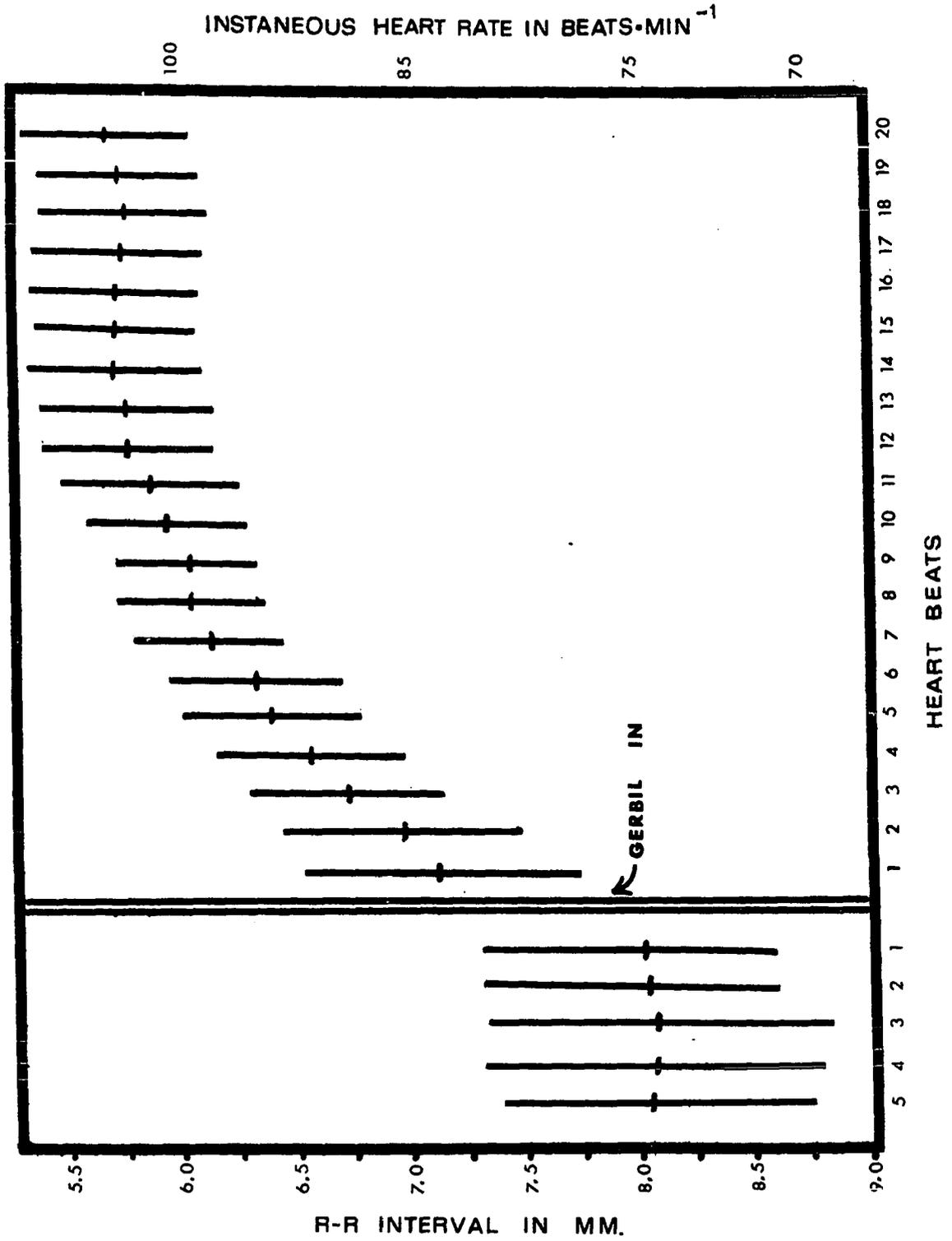
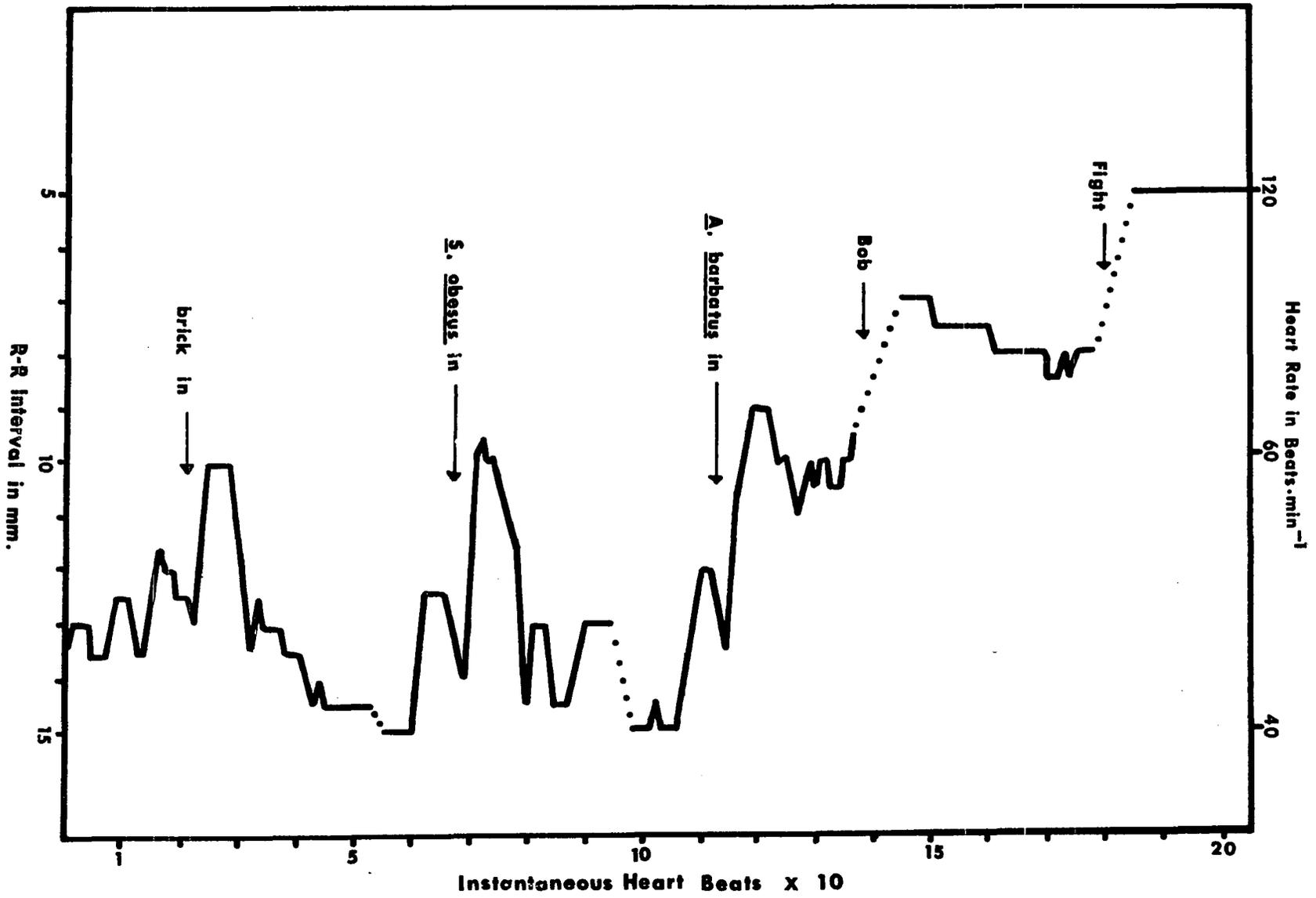


Fig. 7. Instantaneous heart rate of a single Amphibolurus barbatus presented with several different stimuli. The instantaneous heart rate is expressed in $\text{beats}\cdot\text{min}^{-1}$ and as R-R interval in mm at $10\text{ mm}\cdot\text{sec}^{-1}$.



beats. Introducing a conspecific had a considerable effect on the cardiac performance indicated by the high heart rate and the long duration of this cardioacceleration. One can assume some degree of stimulus discrimination on the part of the lizard.

The cardiac parameters of conspecific interaction (Chapter V) and discrimination (Chapter VII) are explored in greater detail.

CHAPTER V

THE RELATIONSHIP OF DISPLAY TO ECG CHANGES IN

AMPHIBOLURUS BARBATUS

Most experimental work with aggressive displays (Hunsaker, 1962; Jenssen, 1969) involved studies of the approach responses either to playback of filmed displays or to cam-driven bobbing models. That physiological parameters are more sensitive to behavioral phenomena than observable indicators such as approach response or other movements has been demonstrated (Chapter IV). The work of Thompson et al. (1968a, 1968b) on starlings, and of Kagan and Lewis (1965) on human infants has indicated the sensitivity of cardiac indicators of behavioral stimuli.

Techniques developed by Carpenter and Sassaman (1968) permit establishment of the precise temporal relationships between displays of a large lizard and the animal's heart rate, or the heart rate of a second lizard observing the display of the first. I have attempted to utilize these techniques to look at the quantitative nature of heart-rate change relating to displays in lizards by observing the heart-rate changes in both dominant and subordinate during the display of the dominant.

Procedures

Two adult Amphibolurus barbatus hand reared in the laboratory from eggs were quite tame and accustomed to handling. Acclimatization reduced

heart-rate changes resulting from stress due to placing electrodes, thermistors, and accelerometers on and in the animals.

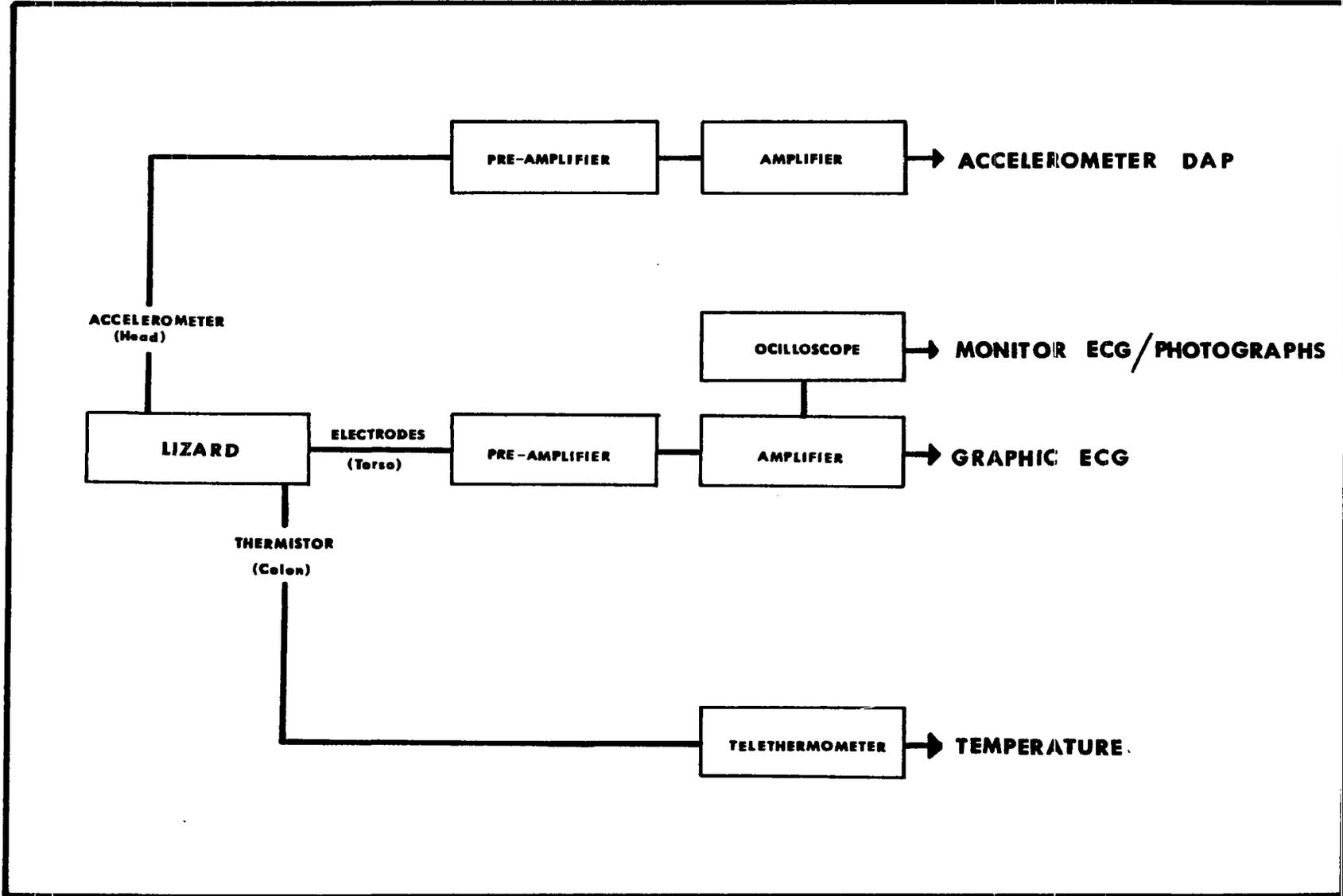
Both animals were housed separately in 5 ft² galvanized steel chambers, each with a glass front and a sand bottom. The top of each chamber was open with a 300 watt heat lamp aimed at the center from one upper corner. The relative social positions of the two animals had already been determined by observing their interactions for several weeks in a 15 x 15 ft enclosure. During this period, the animal subsequently designated as the dominant consistently displayed aggressively toward the animal designated as the subordinate, causing the latter to retreat and attempt to jump out of the enclosure. Trials were conducted with both animals in the home enclosure of the dominant.

The recording instrumentation was designed primarily to record the vertical acceleration of the dominant animal's head during his display, and only secondarily to record electrocardiograms. Recordings were taken from the dominant and the subordinate during different trials.

The set-up used (Figure 8) recorded the vertical pattern of the display of the dominant through a Grass model 7PIA low-level direct-current preamplifier with driver amplifier (Chapter III). The accelerometer, weighing 10 grams, was firmly affixed to the head of the lizard with white glue. The accelerometer tracings (Figure 9) represent positive and negative acceleration of the head of the lizard rather than actual movement.

Accelerometer, saddle, and wires had no apparent effect upon the temporal pattern of the display. Motion pictures taken with an Arriflex 16 mm camera at 25 frames·sec⁻¹, of a lizard bobbing both with and with-

Fig. 8. Diagrammatic representation of parameters recorded from displaying Amphibolurus barbatus.



out an attached accelerometer, showed no change in the temporal relations of the display units, or in the lengths of the units resulting from attachment of the accelerometer.

A thermistor was inserted several centimeters into the colon. Grass platinum needle electrodes were inserted subdermally in the d.v. configuration in either dominant or subordinate. The dominant always had the accelerometer attached to record displays. All wires coming from one animal were fastened with masking tape and tied to a loop of string suspended over the center of the cage before passing to the polygraph and telethermometer and enabling the lizards to move freely. Recordings were made only during the afternoon at least 24 hours after the animals had been fed.

The animals were wired at least one hour before trials. When ECG's were taken from the subordinate, he was placed alone in the enclosure of the dominant to acclimate for several hours to the novel surroundings and reach a basal ECG rate. The dominant was then returned to his home cage with the subordinator present to begin trials.

Results

There are two basic types of aggressive displays in Amphibolurus barbatus (Carpenter et al., 1970). Only the challenge displays, in which there is a deep bow preceded by a series of deep push-ups and followed by a series of successively shallower push-ups (Figure 9), were examined in relation to heart rate. Among the series of challenge displays there was variation of display intensity in terms of the number of display units both preceding and following the deep bow, resulting in differing lengths of time involved in the displays analyzed. To correct for this difference

Fig. 9. The challenge display of Amphibolurus barbatus as recorded cinematographically, as recorded by accelerometer, and as divided for display analysis.

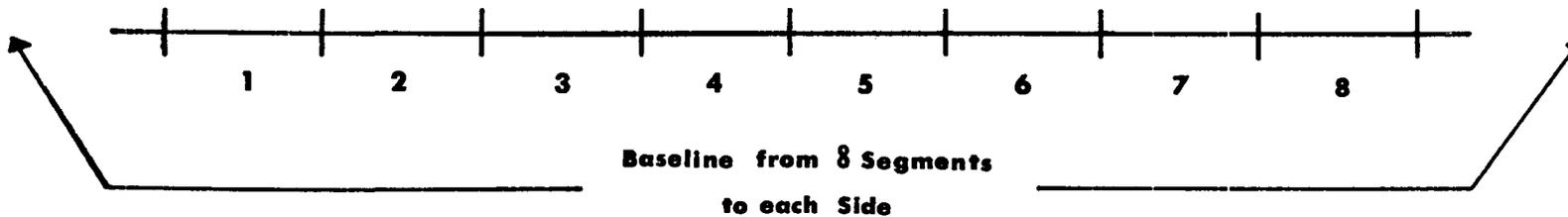
**Cinematographic
Analysis**



**Accelerometer
Tracing**



**Analysis
Segments**



during analysis, heart rate was determined on the basis of length of the display rather than absolute time units.

Analysis of Heart Rate

Mean heart rates of dominant and subordinant animals were examined before, during, and after the display of the dominant. Each display was divided into four equal parts, and heart rate was determined for each. Heart rates were also determined for the two $\frac{1}{4}$ -display units or intervals immediately preceding and immediately following the display for a total of 8 consecutive $\frac{1}{4}$ -display units. The heart rates were compared to a basal rate obtained by taking an average for units preceding and following the display (Figure 9).

Variables Affecting Heart Rate

Three major sources of variation influence heart rate: individual differences between the animals, differences between the actual displays, and the differences due to the effect of the eight $\frac{1}{4}$ -display segments. Data were analyzed by means of a three-factor partially nested ANOVA (Sokol and Rholf, 1969) in which the 19 separate displays were nested within the two individual lizards (Table 2).

The first order variations were all significant ($p < .001$). Whether significance of the variation due to the individual lizards resulted from factors related to the relative social positions of the two animals, or to individual variation, cannot be definitely determined from these data. However, the base rate of the subordinate was higher ($p < .001$) as expected since the subordinate was in a novel and potentially hostile situation.

The effect of the display as reflected in the separate display

Table 2. ANOVA table showing the variance components of animals, display segments, and displays in Amphibolurus barbatus.

<u>Source of Variation</u>	<u>df</u>	<u>Sum of Squares</u>	<u>Mean Squares</u>	<u>F</u>
A. Lizards	1	66042.276	66042.276	83.24***
B. $\frac{1}{4}$ -Display Segments	7	7453.637	1064.805	519.42***
C. Displays (Nested)	36	28563.304	763.452	386.76***
Lizards-Segments (Interaction)	7	1392.393	198.913	96.96***
Segments-Displays (Interaction)	252	516.966	2.051	

*** $p < .001$

segments was again expected in both the dominant and subordinate animals. At least two factors were probably involved in the increase in heart rate of the dominant during the time-course of the displays. The animal was vigorously exercising during his display. Increased tissue oxygen requirements would lead to an increase in cardiac output with an increase in heart rate and probably stroke volume. Because of the agonistic nature of the display, there was probably a strong component resulting from adrenal medullary output of catecholamines acting to accelerate the heart rate. These same two factors most likely play similar roles in the subordinate, where, rather than overt muscular exercise, isometric tensing of muscles for fight or flight occurred. In the subordinate the heart rate increase due to action of adrenal medullary hormones probably accounted for a greater percentage of the total heart-rate increase than in the dominant. The exact quantitative nature of these two factors in either the dominant or the subordinate is unclear and should be investigated further.

The third source of first-order variation was due to the added component of the individual displays themselves, nested within the first factor, the lizards. The displays were nested, since any variation in them may be caused in part at least by the two separate lizards involved. The analysis was designed to separate the variation in heart rate due to the displays themselves from that due to differences in the lizards. Again, significance of the nested display component ($p < .001$) indicates significant variation in the heart rate of the two lizards from one display to the next.

These differences in heart rate may have arisen from such factors as

differing base heart-rate levels, differences in core body temperatures, time of day, or any other environmental variable.

A significant ($p < .001$) second-order interaction between lizards and segments indicates that changes in heart rate during the eight $\frac{1}{4}$ -display segments occurred differently for the two lizards (Figure 10). The mean heart rate in each of the eight $\frac{1}{4}$ -display segments and the base heart rate for each of the two lizards were compared separately in figure 10. The heart rate of the subordinate was higher at all times than that of the dominant; indeed the dominant's heart rate at its maximum did not reach the base level of the subordinate, this despite the seemingly vigorous muscular exertion often observed in the dominant animal during display. By the last $\frac{1}{4}$ -display segment, the heart rate of the subordinate decreased to a level closer to that of its base rate than had the dominant's. Again, more data is necessary to determine whether this is due to social position or merely to individual variation.

In figure 11, the same heart-rate data during the display are shown, but in terms of mean heart rate for each $\frac{1}{4}$ -display segment, presented as a percentage of base heart rate rather than in $\text{beats} \cdot \text{min}^{-1}$ as in figure 10. By the final $\frac{1}{4}$ -display segment, heart rate of the subordinate returned to the base level, while that of the dominant was still significantly ($p < .05$) above the base. During display, there was a greater increase in heart rate of the dominant ($p < .05$) and it preceded the heart-rate increase of the subordinate. At the onset of the display, the dominant's heart rate increased immediately, reflecting the effect of muscular exertion and increased autonomic activity due to behavioral factors. At the end of the display, however, the dominant's heart rate

Fig. 10. Heart rate in $\text{beats}\cdot\text{min}^{-1}$ of both dominant and subordinate Amphibolurus barbatus before, during, and after display of the dominant, as in figure 3. Solid error bars indicate the dominant.

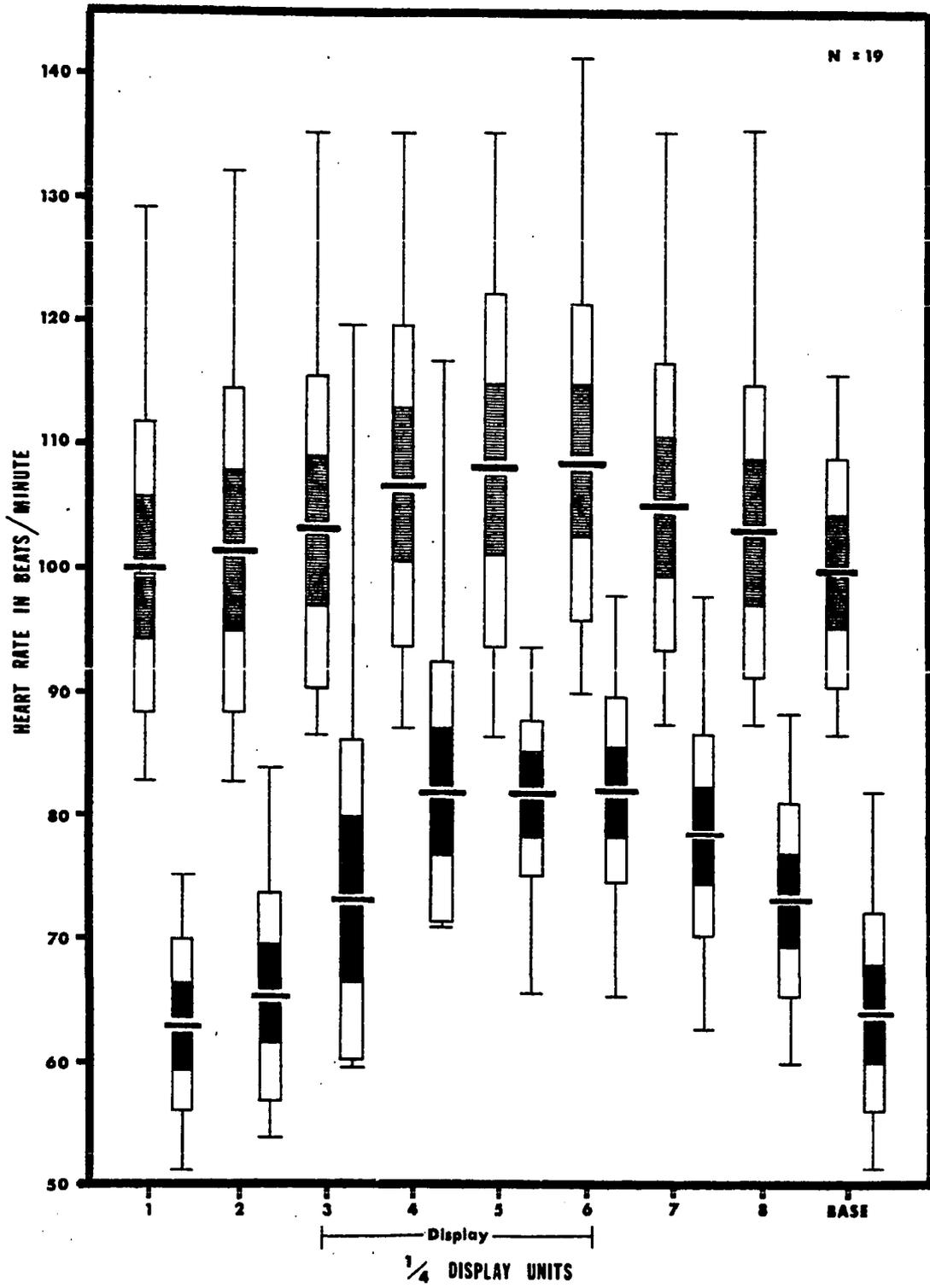
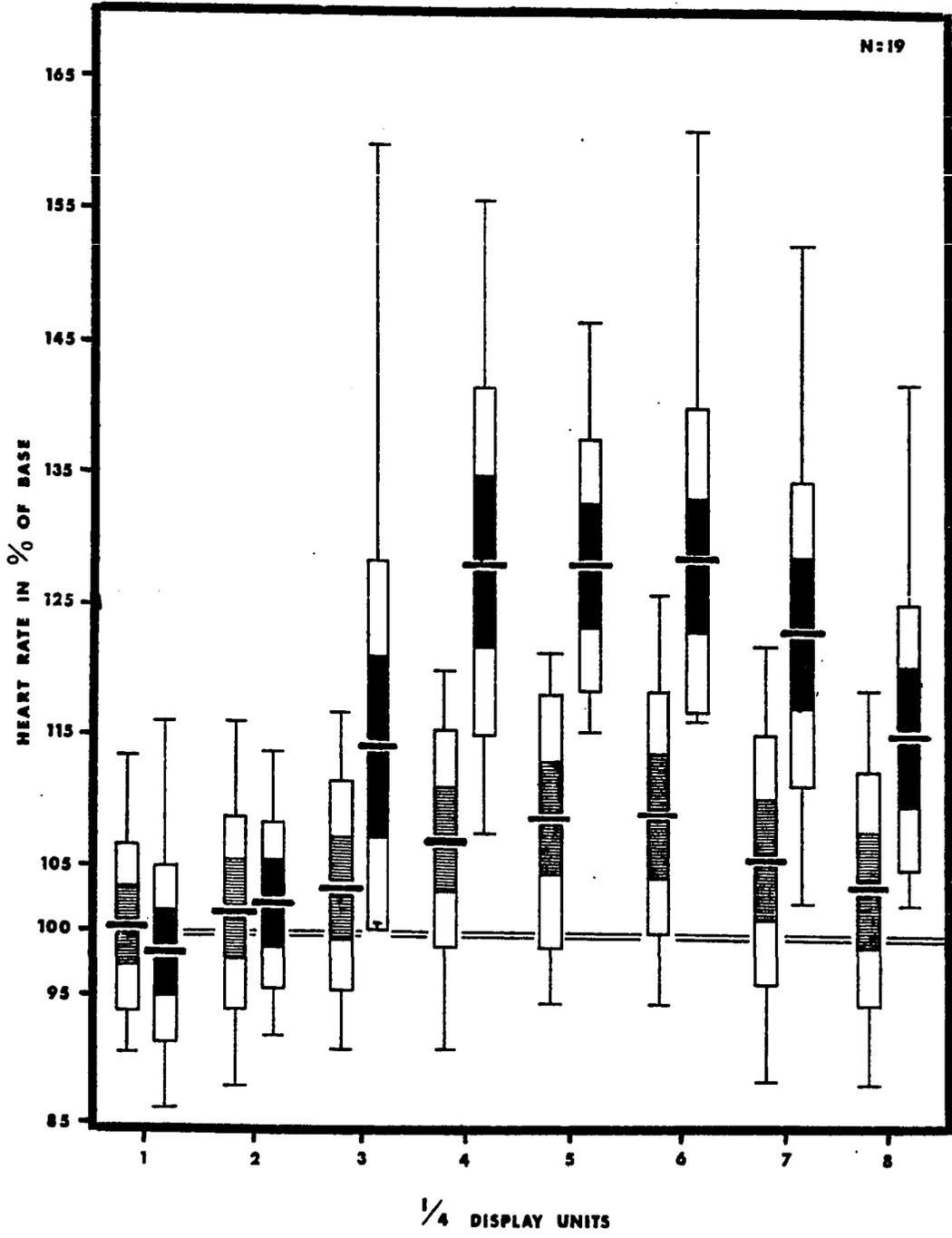


Fig. 11. Heart rates of dominant and subordinate Amphibolurus barbatus before, during, and after the display of the dominant, expressed as a per cent of the base heart rate. Statistics of dispersion are as in figure 3. Solid error bars indicate the dominant.



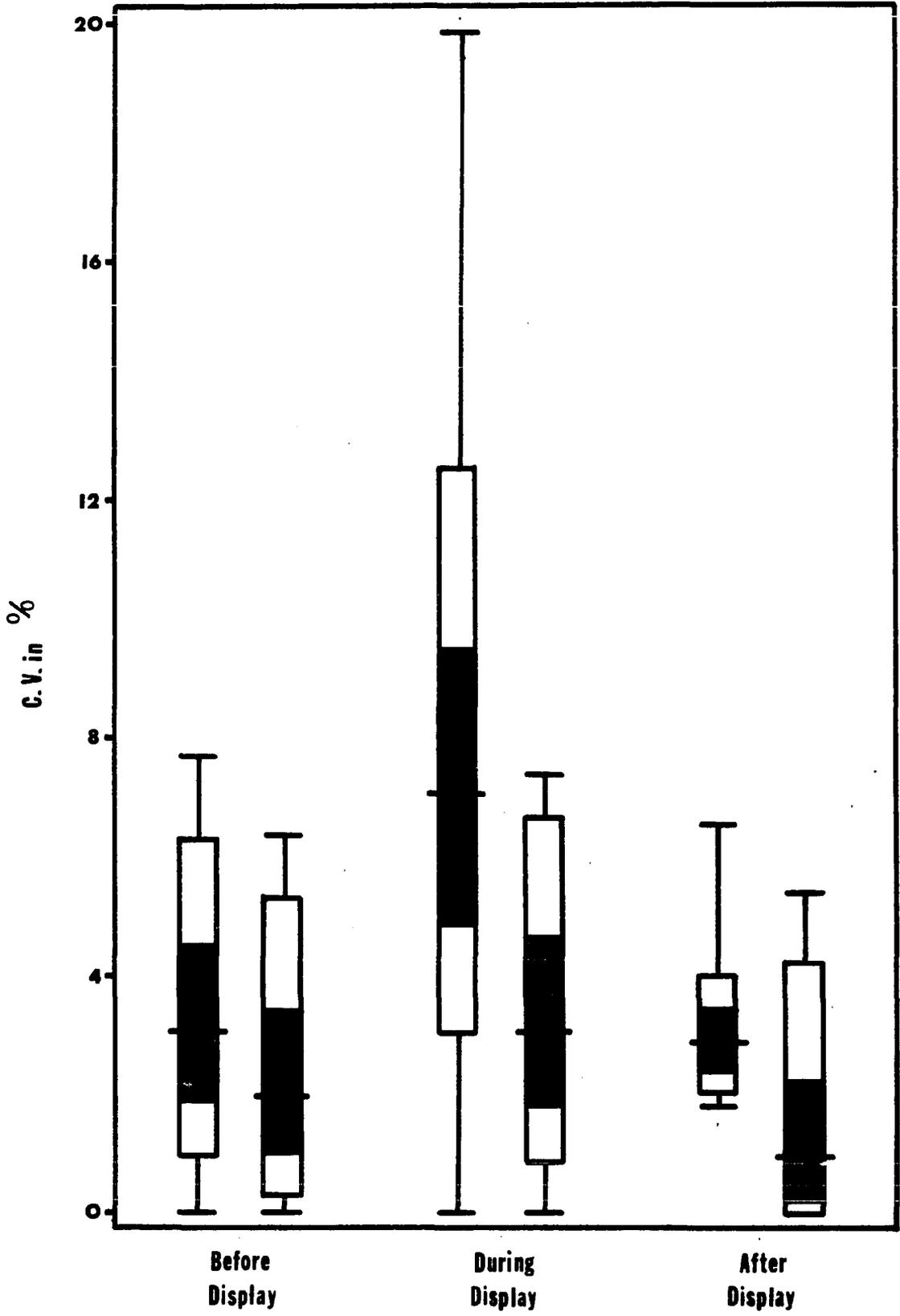
tapered off slowly toward the base level but did not reach it by the final $\frac{1}{4}$ -display segment sampled ($p < .05$). Heart-rate increases during display were not significant in the subordinate, but were definite and occurred in display segments four through seven. This delay represented a time lag of only a few seconds, occurring at both the initiation and the termination of the dominant's display. It follows that the heart rate of the subordinate is a dependent variable weakly indicative of biologically important aggressive signaling from the dominant animal.

Heart Rate Variability During Display

Another question arises concerning the differences in variability of cardiac activity of the dominant and the subordinate before, during, and after the display of the dominant. I examined the instantaneous heart rates in the ECG recordings of each animal during the 19 displays already analyzed for changes in instantaneous heart rate. The instantaneous heart rate was obtained by determining the distance between successive QRS complexes to the nearest 0.5 mm. These were then tabulated in terms of the last 10 heart beats preceding the first $\frac{1}{4}$ -display unit, the first 10 heart beats during the display itself, and the first 10 heart beats following the termination of the display. A coefficient of variation was obtained for each of these samples, normalized as described earlier (page 18) and analyzed for the pre-display, display, and post-display heart-rate variability for both animals (Figure 12).

The greater variability of heart rate in the dominant ($p < .05$) reflects the change in rate that occurred as the dominant's heart rate increased during display and then declined after its termination. Possibly the great difference in variation in heart rate between dominant and

Fig. 12. Coefficients of variation of heart rate for dominant and subordinate Amphibolurus barbatus before, during, and after the display of the dominant. Coefficients of variation were based on 10 beats per segment for each of the 19 trials. Statistics of dispersion are as in figure 3. Solid error bars indicate the dominant.



subordinate during the display was due to the added need for oxygen, indicating that muscular exercise or increased oxygen demand induces an increased heart-rate variability.

A three-factor partially nested ANOVA was performed on these data. The three factors were the lizards, the segments (pre-display, display, and post-display), and the 19 displays nested within the lizards (Table 3). Significant variations ($p < .001$) due both to the individual animals and to the three segments of display and non-display indicate differences in variability in the two animals and between the segments. Non-significance of the second-order interaction ($p > .05$) between lizards and segments indicates that heart-rate variability change during the three segments is independent of the animal.

Significant differences ($p < .001$) in heart-rate variability during the display compared to the preceding and following segments (Table 4) indicate that heart-rate variability is indeed greater during the display than before and following it. This reflects change in heart rate at the onset of the display, and possibly greater excitability during the display.

There was no difference in heart-rate variability between the pre- and the post-display segments (Table 4), indicating that the variability change occurs only during the display, and decreases to the pre-display level soon after termination of the display. In several cases, particularly in the subordinate, the heart rate was constant (coefficient of variation = 0) during the pre-display or the post-display or both.

Intensity of Display and Heart Rate

The intensity of the challenge displays for which heart rates were

Table 3. ANOVA table showing variance components of heart-rate variability due to animals, segments (pre-display, display, post-display) and displays in Amphibolurus barbatus.

<u>Source of Variation</u>	<u>df</u>	<u>Sum of Squares</u>	<u>Mean Square</u>	<u>F</u>
Lizards	1	407.433	407.433	21.49***
Segments	2	512.378	256.189	10.10***
Displays (Nested)	36	682.550	18.960	0.75 ns.
Lizards-Segments	2	54.313	27.156	1.07 ns.
Segments-Displays	72	1826.386	25.366	

*** $p < .001$

Table 4. A priori comparisons of heart-rate variability during pre-display, display, and post-display segments in Amphibolurus barbatus.

<u>Pre-display</u>	<u>Display</u>	<u>Post-display</u>	<u>Sum of Squares</u>	<u>Mean Square</u>	<u>F</u>
_____	_____	_____	471.658	25.37	18.59***
└-----┬-----┘			40.720	25.37	1.61 ns.

*** $p < .001$

Table 5. Correlation coefficients between display intensity and several measures of heart-rate change in dominant and subordinate Amphibolurus barbatus.

	<u>Absolute Maximum Heart Rate</u>	<u>Maximum Increase Over Base</u>	<u>Maximum-Minimum Difference</u>
Dominant	0.125 ns.	0.402 ns.	0.257 ns.
Subordinate	0.195 ns.	0.113 ns.	0.292 ns.

recorded varied from 9 to 16 display units. There was, however, no correlation (Table 5) between display intensity measured in terms of the number of units and the absolute maximum heart rate, the difference between maximum and minimum heart rate, or the maximum increase over the base level for either the dominant or the subordinate. Even in the dominant, exercising at different levels of excitement with the different bob intensities, there was no correlation between heart rate and bob intensity. This may be due to the different display intensities having the same actual degree of exercise intensity, or to the heart beating at its maximum rate for the particular temperature involved. There was no attempt to correlate the intensity of the display of the dominant with the rate of decrease in heart rate following termination of the display.

Conclusions

The sensitivity of heart-rate changes to such behavioral phenomena as aggressive displays is indicated in these data (Figures 10 and 11). Several parameters of heart-rate change serve as dependent variables indicative of socially important behavioral actions, including change in heart rate itself and change in heart-rate variability.

Several other correlations could be made between ECG and behavior. It is possible that the temporal relationships between the ECG component waveforms change with behavioral changes, but my data do not lend themselves to such an analysis. Another approach would be to attempt to relate changes in the amplitude of the ECG waveforms to behavioral changes.

CHAPTER VI

HEART RATE AND SOCIAL POSITION IN AGAMA AGAMA

In previous chapters I have shown increases in heart rate of lizards associated with social and non-social stressors. Chapter V concerns the heart rate of only two individuals in a social situation, but poses additional questions.

1) Is there any consistent correlation to be made between heart rate of lizards and their social position within a group hierarchy?

2) If this relationship does exist, is it stable or does it change as the lizards remain for a time in the social grouping?

Four male and two female Agama agama were obtained commercially. This species was chosen for its availability and because the dominant males are easily recognized by their bright coloration (Harris, 1964). The males were immediately measured and paint-marked for identification. Although this group of six was kept together, ECG's were recorded only from the four males.

Upon receipt, the base heart rate of each male was determined as outlined below. All of the lizards were then placed in a 16 x 12 ft Sheerer-Gillett environmental chamber with sand floor and rocks scattered about for cover. The lizards were maintained at a temperature range of 25° to 35°C and a light-dark cycle of LD 10 $\frac{1}{2}$: 13 $\frac{1}{2}$ centered at 13:45

hours, Central Standard Time.

Heart Rate

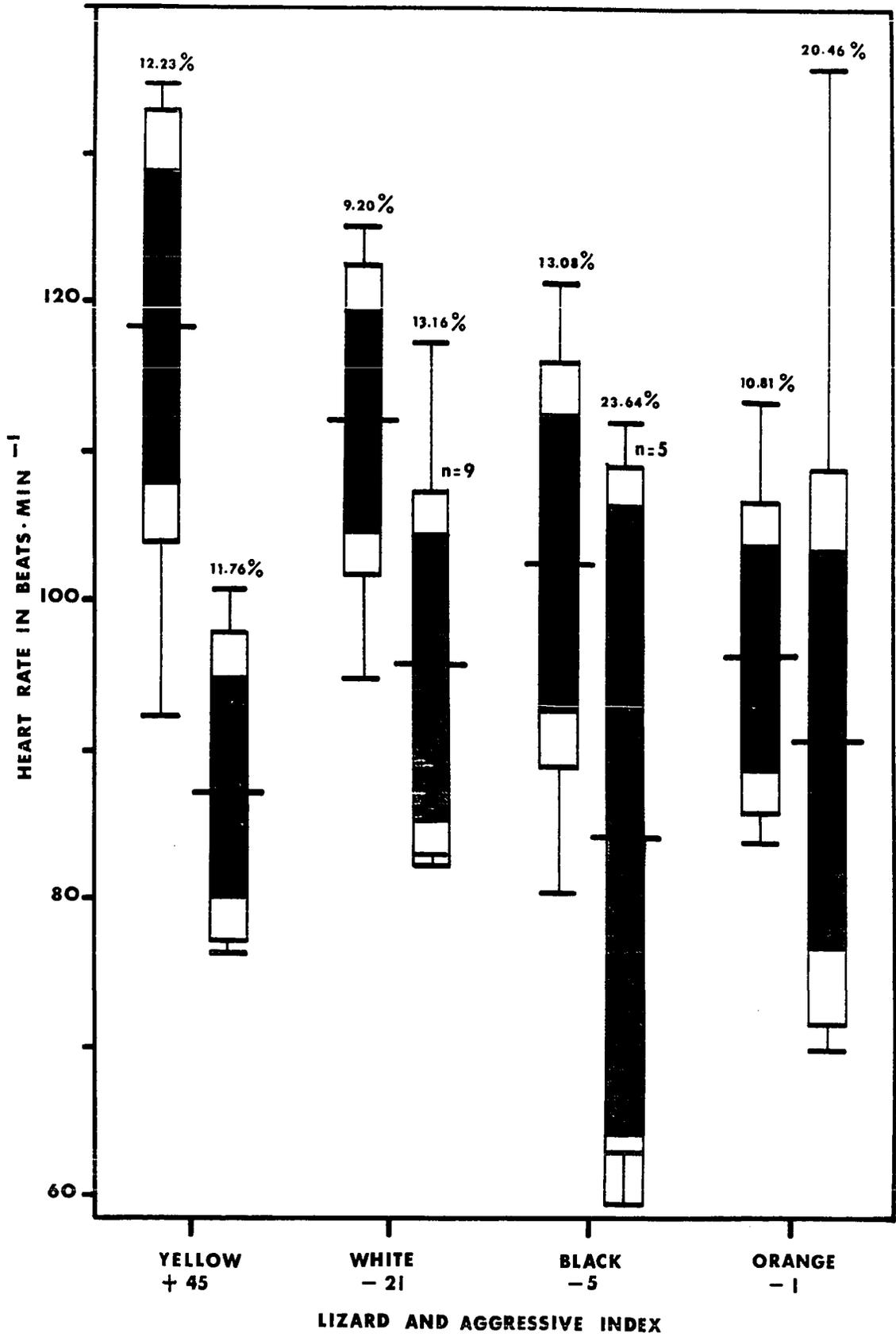
Heart rate was determined using the d.v. platinum electrode system described (page 9). The lizards were removed from the chamber in random order each day of recording and weighed, electrodes attached, and a thermistor was placed in the lower colon to monitor body temperature. They were then placed in a 10-gallon aquarium and allowed to acclimate for 30 minutes. Just prior to recording, a heat lamp was activated several feet above the aquarium, and the ECG was recorded as the animal's colonic temperature increased from 30°C to 31°C. The temperature was recorded at $\frac{1}{2}$ -degree intervals during the ECG recordings. Heart rate in $\text{beats}\cdot\text{min}^{-1}$ was determined by doubling the number of R-R intervals within a single 30-second segment at 30, 30.5, and 31°C. The mean of these three was considered the heart rate for that day.

Heart rate was determined daily for each of the four lizards during a 10-day period starting two weeks after the lizards were initially grouped. This was followed by a two-week period of no recording, and a subsequent second 10-day recording period. This second recording period was initiated to determine any long-term changes in heart rate due to the social grouping (Figure 13).

Social Behavior

The lizards were observed several hours daily for their first 17 days in the chamber. Social encounters and general behaviors were noted. Times of observation varied, but were usually in the morning. Observations through a one-way mirror followed a brief entrance into the chamber to check air temperature.

Fig. 13. Heart rate in $\text{beats}\cdot\text{min}^{-1}$ fo four male Agama agama for two 10 day recording periods. Statistics of dispersion are as in figure 3. Solid error bars represent the first recording period. Unless otherwise indicated, $n=10$. The coefficient of variation in per cent is indicated above each bar.



Based upon an aggressive index (Table 6), the yellow male was assumed the dominant individual in the group and the white male the subordinate. The orange and black males were classed as neutral individuals.

Although detailed observations of social behavior were not conducted during the second 10-day recording period, spot checks indicated no apparent change in the behavioral relationships between any of the animals. Therefore I assumed that the original social relationships remained unchanged.

Discussion

Yellow Male

During the initial recording period, the dominant male (yellow; A.I. \pm 45) had the highest heart rate (Figure 13). Significantly greater than both the orange and black males ($p < .05$), this may reflect effects of social stress involved in the initial establishment and maintenance of dominance. During the second recording period, the heart rate did not differ significantly from the rest of the group. It had, however, decreased significantly ($p < .05$) from the higher rate of two weeks previously, indicating a well established dominant status that no longer subjected this animal to a high level of social stress.

Orange Male

The orange male, with an aggressive index of -1, was considered a neutral animal in the hierarchy. During the first 10-day recording period, his mean heart rate was lower ($p < .05$) than that of the dominant. This rate had not changed significantly two weeks later during the second recording period, nor did it differ from that of any of the other males.

Table 6. Members of Agama agama social grouping arranged in order of descending social rank. The aggressive index was derived by subtracting the number of aggressive attacks received from the number of aggressive attacks initiated. The data from the two females are lumped.

Lizard	Aggressive Attacks <u>Initiated</u>	Aggressive Attacks <u>Received</u>	Aggressive <u>Index</u>
Yellow Male	47	2	+45
Orange Male	4	5	-1
Black Male	1	6	-5
White Male	1	22	-21
Females	5	23	-18

The only noticeable difference between the two periods in this individual was found in the day-to-day variability in mean heart rate as reflected in the coefficient of variation.

Black Male

This individual was also neutral (A.I. = -5) with no significant change in mean heart rate between the two recording periods. Since this lizard died after five days of recording during the second recording period, the great increase in day-to-day irregularity of heart rate during the latter recording period as reflected by the coefficient of variation may have resulted from failing health.

White Male

The white male, with an aggressive index of -21, was considered the subordinate individual of the group. During the first recording period, the heart rate of this individual was higher ($p < .05$) than during the second recording period. Although this lizard died after nine days during the second recording period, there was only a slight increase in the coefficient of variation from the first to the second recording periods.

General Observations and Summary

The two socially active individuals with the highest and lowest aggressive indices both displayed significant decreases in mean heart rate between the two recording periods. The most likely reason for this is that by the second recording period, the animals were firmly established in their social positions, and further maintenance resulted in less social stress than did its establishment. This may also explain the constancy in day-to-day variability of mean heart rate for these two

individuals between the two recording periods, during the second recording period there being less variability in the day-to-day social stress with social position now established. The two individuals with the neutral aggressive indices, the black and the orange males, displayed no significant change in mean heart rate between the two recording periods. They both, however, did show a considerable increase in day-to-day variability of heart rate during the second recording period. These phenomena are best explained by the lack of certainty in the social position of these two socially neutral individuals.

CHAPTER VII

SIGN-STIMULUS COMPONENTS OF AGGRESSIVE DISPLAY OF

GROTAPHYTUS COLLARIS

The next logical question to ask is if cardiac responses in lizards can be used as a tool to demonstrate behavioral phenomena. In this chapter I have used changes in cardiac activity as the dependent variable in order to examine the relative importance of body colors and display action pattern as aggressive signals in lizards.

Due to its bright coloration, simple display, and relative ease of supply, Crotaphytus collaris was used. The turquoise color and black collar undoubtedly serve as camouflage, while the bobbing motion of the bright orange head during the aggressive display makes the animal more conspicuous, at least to the human observer.

In the present experiment, naive animals were presented with models representing conspecific males or a male fence lizard (Sceloporus undulatus). Cardiac responses of the lizards to the models were then monitored in an attempt to use differential cardiac response to determine the mode by which aggressive signals are transmitted by this species. This assumes, of course, that an aggressively significant signal will induce a cardiac change in the receiver animal. Evidence to support this assumption has already been presented.

ECG Response to Models

Animals

Only recently collected (and thus assumed healthy) males were used, the majority from Kiowa County, Oklahoma, in the region circumscribed by a triangle connecting the towns of Rocky (Washita County), Saddle Mountain, and Roosevelt.

Each animal was toe-clipped and colonic temperatures were taken at capture. On return to the laboratory, each was weighed, measured, and soaked overnight in an antibiotic solution before a one week acclimation period in a 16 x 12 ft environmental chamber with sand floor and various rocks for shelter. Food in the form of crickets and insect sweepings, and water, were provided ad libitum every other day. The chamber was maintained at a temperature of 20°C., a relative humidity of 30-50%, and a photoperiod of LD 12:12 centered at 13:00 hours Central Daylight Time.

Recording Procedures

Models -- Five latex rubber models were used, four representing modified Crotaphytus collaris and one representing Sceloporus undulatus in size, posture, and coloration. The Crotaphytus models were painted as follows: turquoise body, orange head; turquoise body, orange head, double-black collar; all orange; and all turquoise. All made from the same mold, the Crotaphytus models were 24 mm long and 5.5 cm high with arched back and a laterally compressed configuration to represent the aggressive posture of the species. Four lizards were run with each model.

Recording -- The lizards were removed singly from the chamber for ECG recordings and weighed. After brass-coated safety-pin electrodes were attached in the d.v. configuration, the lizards were placed in a 3 ft³

chamber with screen top and glass front. A thermistor probe at the bottom of the cage monitored temperature. The glass front of the chamber was masked with cardboard except for a small square covered with one-way glass, placed at an angle to prevent the lizard from responding to his own reflection. The room in which recordings were made was kept relatively dark, and light was kept out of the chamber except during trials.

Recordings were made with a Grass Polygraph between 12:00 and 6:00 PM Central Daylight Time from June through September. Animals were placed singly with ECG leads attached in the darkened recording chamber and allowed to acclimate approximately one hour until a steady ECG was maintained. One minute stimulus presentations were effected by activating a 300 watt sunlamp one meter above the chamber floor with the model in the chamber. Using a variable transformer, the light was turned to maximum brightness over a two-second interval avoiding undue alarm on the part of the lizard by the sudden onset of light. As a control for the effect of light, the light was turned on for two consecutive one-minute trials with a five-minute period between the light trials. With the chamber dark, the model was positioned in the middle of the chamber floor. After 15 minutes to allow the lizard to calm down from this disturbance, the model was presented for successive one-minute trials interdicted with five-minute inter-trial periods. Model presentation was effected by turning on the light within the chamber. Nylon mono-filament fishing line was attached to the model, enabling manipulation to imitate the display of Crctaphytus. The short hopping motions of the aggressive male were easily imitated by short rapid tugs on the mono-filament. A

series of one-minute trials was then repeated with the bobbing model (Figure 14).

Analysis

The ECG's register individual heartbeats as R-waves; relevant portions were converted to instantaneous heart rate by measuring R-R intervals. Each trial was divided into nine 10-second continuous segments: two pre-trial segments (these then averaged as a baseline) six trial segments, and one post-trial segment (Figure 14).

Following techniques used by Kagan and Lewis (1965), cardiac lows, or heart-rate minima, were determined. This entailed taking the five slowest heart beats in each of the 10-second segments and converting the average of these five R-R intervals into $\text{beats}\cdot\text{min}^{-1}$. The data for all animals in each model presentation were pooled, and presented as a per cent increase over the base heart rate (Figure 15).

In order to determine those factors resulting in cardioacceleration, a three factor ANOVA was conducted in the heart-rate minima determined above. The mean heart-rate increases for each lizard were lumped for each repeated trial with both bobbing and non-bobbing models. The only significant factor was the motion of the models (Table 7; $p < .05$).

Results

Each series of presentations is comprised of three main segments: the initial two presentations of light alone action as a control for the effects of light on cardiac response; the series of one-minute trials with a motionless model: and the series of one-minute trials with the model bobbed to imitate the species specific display of Crotaphytus collaris.

Fig. 14. Block diagram of experimental trials of model presentation of Crotaphytus collaris.

ANALYSIS OF TRIAL SEGMENTS

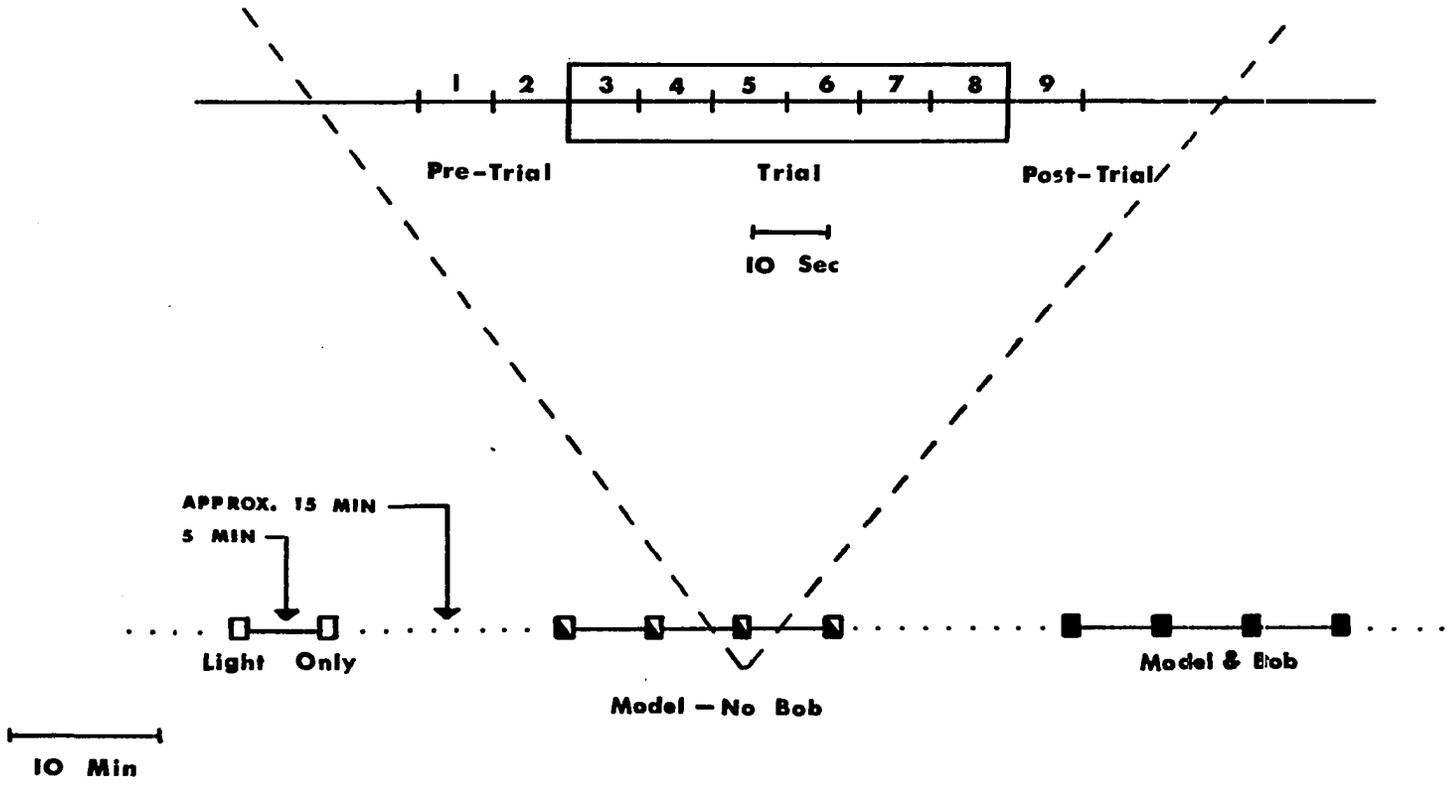


Table 7. ANOVA table showing variance components of heart-rate change due to models, treatments (non-bobbing vs. bobbing models), and repeated trials in Crotaphytus collaris.

<u>Source of Variation</u>	<u>df</u>	<u>Sum of Squares</u>	<u>Mean Square</u>	<u>F</u>	
Models	4	302.376	75.594	1.789	ns.
Treatments	1	233.337	233.337	5.521	p<.05
Trials	3	397.168	132.389	3.132	ns.
Models-Treatments	4	371.908	92.977	2.200	ns.
Models-Trials	12	443.644	36.970	.875	ns.
Treatments-Trials	3	324.164	108.055	2.557	ns.
Models-Treatments-Trials	12	507.169	42.264		
Total	39	2579.766			

Cardiac responses to the light alone can be lumped for all of the model presentations together. As indicated in figure 15, there is heart-rate elevation during the first light presentation, but not during the second. This elevation is indicative of a rapid habituation to the light stimulus by the second trial. It is assumed that the light did not have any cardioacceleratory effect during further trials.

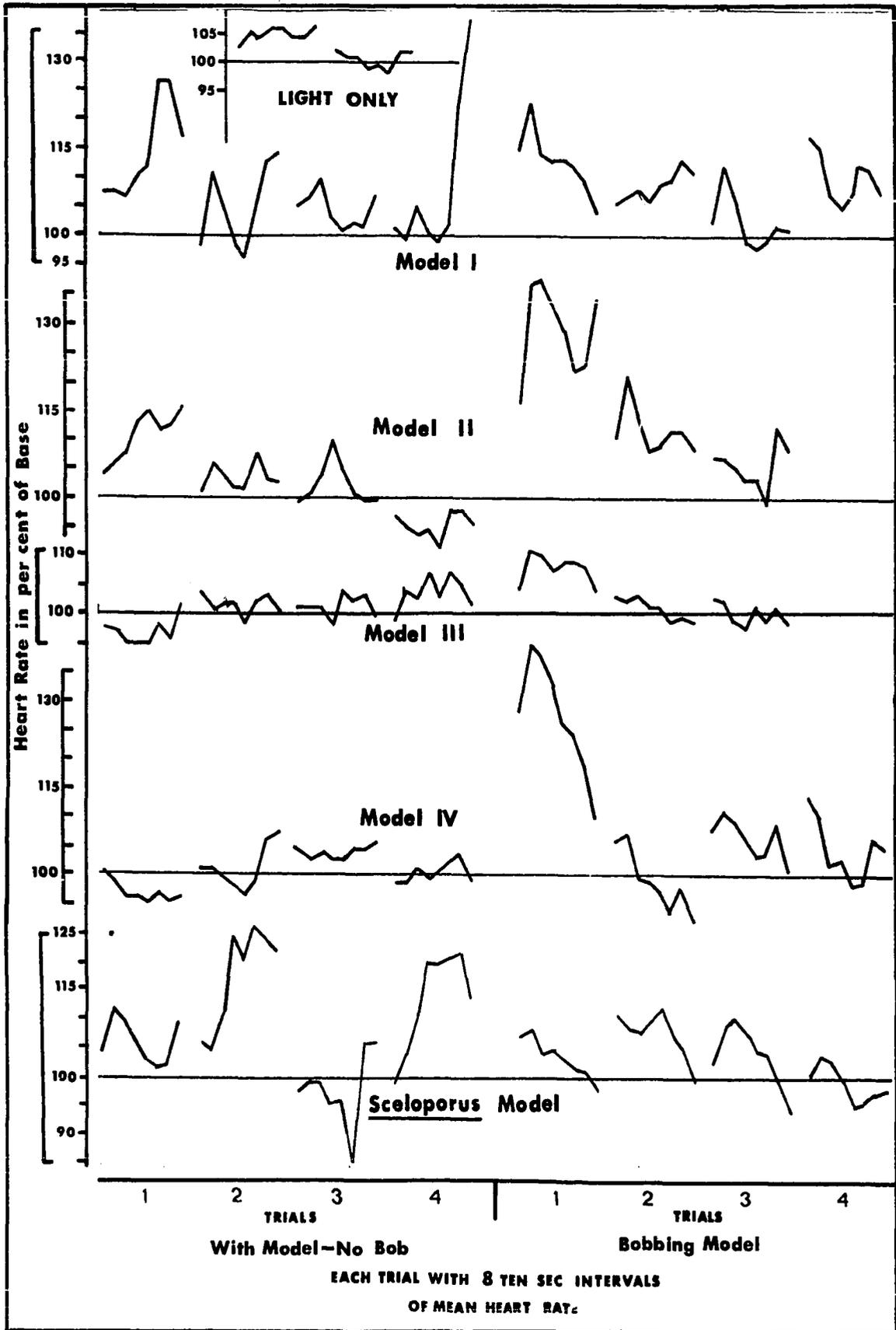
From these data, one may make several observations.

1) The initial responses of the lizards to models painted with orange head and turquoise body (Models I and II) is one of cardioacceleration, but the response quickly habituates (Model I) or turns into cardiac deceleration or attention (Kagan and Rosman, 1964). These responses indicate that the orange head contrasting with the turquoise body serve to communicate an aggressive signal to the conspecific. The immediate physiological response on the part of the conspecific receiver to this aggressive signal is one of rapidly declining cardioacceleration when the model remains motionless. It would seem that contrast of color is important here.

2) The only other instance of marked cardioacceleration to a motionless model is to that of Sceloporus. In this case, heart rate displays a tendency to increase during the individual trial with a motionless model. Perhaps in this case the stimulus of the model is foreign enough to create anxiety rather than attention in the receiver Crotaphytus and thus leads to cardioacceleration.

3) Cardioacceleration resulted when the models were caused to bob. In the case of the orange model (Model III), the cardioacceleration was not great, resulting only in the first bobbing trials. In the case

Fig. 15. Mean cardiac reaction by trial and stimulus for Crotaphytus collaris expressed as a per cent increase over base rate for heart-rate minima.



of the turquoise model (Model IV), there was a large increase in heart rate during the first trial with a bobbing model. Again there was rapid habituation, such that the remaining bobbing trials deviated little from the base rate. In Models I and II, this habituation was not as marked, indeed, in the case of Model I, the final bobbing trial still resulted in cardioacceleration.

4) It is evident that while a lizard may respond with cardioacceleration, probably due to anxiety when the model is first moved, a continuation of that response in later trials requires the proper assemblage of sign-stimuli components in the form of contrasting head-body coloration and movement acting together to result in aggressive communication.

Field Response to Models

Overt response orientations of lizards in a captive situation have been demonstrated (Harris, 1964; Jenssen, 1969). It could be argued, however, that the cardiac responses of Crotaphytus to models in the laboratory situation may be more an indication of the stresses of the artificial situation of the laboratory environment than of reality itself. Indeed, the animals rarely made any detectable overt response to the models presented in the chamber.

Several questions can be answered using models of aggressive Crotaphytus in the field.

- 1) Do territorial male animals in the field respond aggressively to conspecific models placed conspicuously within their territory?
- 2) What type of response, if any, is elicited by the models; do the lizards passively watch the model, or do they actively attempt to

assert themselves, or even to dispose the model?

In an effort to examine reactions of territorial male Crotaphytus to a conspecific model, limited field work was conducted during the summer of 1970 as a secondary objective during collection trips. To separate collection and observation populations, field observations were carefully restricted to one section of land (Township 7 North, Range 17 West, Section 22) located seven miles west of Gctebo on the southerly side of Oklahoma State Highway 9 in Kiowa County.

Running through the southern half of this section in an east-west direction is a steep escarpment of limestone overlaying red sandstone with a south scarp slope. The many blocks of limestone that have fallen down, as well as the crevices in the limestone itself, provide shelter for an abundant population of Crotaphytus. These factors, as well as the relative isolation from human activity, allow undisturbed observations.

Only a few observations were made with Models I and III, but they presented evidence that a displaced territorial male will respond to these models.

One observation is particularly noteworthy, and was conducted for more than an hour on 15 June, 1970, in the territory of a male with two observed females. The male was displaced from his territorial post on a large boulder, and Model I was taped firmly in his place. The lizard, meanwhile, ran to another boulder seven meters away and hid under it. Five minutes after I had retired to an observation blind, the lizard left his retreat and approached the model. The approach was cautious at first, but as the lizard drew closer to the model, he arched his back and began to display toward the model in the face-off position described (Carpenter,

1967) for other Iguanids. When no response was forthcoming the lizard approached the model from behind and licked its femoral region several times, then immediately moved away and assumed an alert, aggressive posture while observing the model. It would appear from this behavior that the taste of the femoral region was the final test of whether or not the model presented a threat to the lizard's territorial position.

Summary

Cardioacceleration results in lizards presented with various models, but the most consistent response to motionless models is to those with an orange head contrasting with a turquoise body. A comparison between non-bobbing and bobbing models displayed a significant ($p < .05$) cardioacceleration with bobbing models. These results indicate a possible interdependence of several sign-stimuli; the display, the body posture, and body coloration in signaling aggressive information in this species.

CHAPTER VIII

DISCUSSION

This study has involved three levels of investigation: development of techniques to monitor ECG's of lizards, investigation of some behavioral parameters of ECG changes, and final utilization of these ECG changes to examine the sign-stimuli components of aggressive communication in Crotaphytus collaris.

Throughout, I have interpreted my results assuming the observed cardiac changes to be mediated independently of overt or covert muscular activity via the autonomic nervous system. Smith (1954), arguing that examples of so-called visceral conditioning can be explained as artifacts occurring with skeletal responses resulting from the process of conditioning, would imply that an assumption of independent mediation of cardiac change is incorrect. In an attempt to counter claims that visceral conditioning in reality represents indirect responses due to learning by skeletal elements, Miller and Banuazizi (1968) selectively conditioned rats to alter either the rate of spontaneous intestinal contraction, or heart rate. These results strongly suggest mediation of visceral function within the higher centers of the central nervous system. Data presented in my study further support an independent mediation of cardiac response. If the observed changes in heart rate are indeed simple one-to-one by-products of muscular activity, as Smith (1954) would argue, positive

correlation between heart-rate change and some measure of muscular activity would be expected. There was, however, no correlation at all between level of display intensity and any measure of heart-rate change in the displaying animal (Table 5). It is possible that some higher CNS center exercises differential control over both cardiac activity and display intensity, or that both are mediated by separate centers.

One may also argue that observed changes in heart rate with presentation of stimuli, such as the Crotaphytus and Sceloporus models, represent non-specific physiological orienting responses (Harlow et al., 1971). These orienting responses to a stimulus involve a number of events, including gross orientation toward the stimulus, pupillary dilation, EEG activation, and a briefly interrupted heart rate. The heart rate changes in my study with the models (Figure 15) should be interpreted as representing some function other than a generalized orienting response, as there were rapid increases in heart rate in the animals when presented with the model. The only logical function is a cue function, as I have assumed.

Several problems become evident from the data presented. The use of heart rate as a peripheral indicator of processes within the CNS may allow for the intermediary action of numerous intervening variables that are often too difficult to control. Many of the physical factors affecting heart rate have been discussed (Chapter II). Malmo (1959), however, defends the use of peripheral physiological functions to elucidate central behavioral mechanisms based upon the use of intraindividual rather than interindividual correlations between two physiological indicators of activation, thus correcting for individual variability in the

parameters studies. In order to correct for individual differences in base heart rate (Figure 10 and 13), I have confined my comparisons, where appropriate, to change in heart rate expressed as a per cent of the base rate. Nevertheless, many other variables are not so readily controlled. With lizards, it is often difficult to be certain that the animal is even observing the stimulus.

In conclusion, it can be said that I accomplished much of what I set out to do.

1) Suitable techniques were developed to obtain clear, usable ECG's from unrestrained lizards.

2) Several behavioral parameters were correlated with ECG responses, such as responses to a small mammal, responses of a subordinate Amphibolurus to the display of a dominant conspecific, and the relative heart rates were compared for members of a social grouping of Agama agama. It should be noted that in the latter study, considerable additional data are necessary in order to predict the relationship between social position and base heart rate if such a relationship exists.

3) Changes in heart rate in Grotaphytus collaris in response to models were used to determine that the contrast between the orange head and the turquoise body probably serves as the sign-stimuli component of aggressive communication in a motionless animal, while the movement embodied in the display serves to further excite the observing animal. Additional data are necessary to determine if this additional excitement is due to the motion, or to the specific cadence of the bob, or to the display.

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