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Cluster Analysis of Stress in Rats

by

© Michael Peter Slawnych

A THESIS

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"I refuse to accept the cynical notion that nation after nation must spiral down a militaristic stairwell into the hell of thermonuclear destruction. I believe that unarmed truth and unconditional love will have the final word in reality. This is why right temporarily defeated is stronger than evil triumphant"

Martin Luther King (1929–1968)

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Cluster Analysis of Stress in Rats submitted by Michael Peter Slawnych in partial fulfilment of the requirements for the degree of Master of Science.

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Supervisor

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Date October 4, 1985

For my wife Lynne, with love, without whose undaunted dedication and inspiration it would not have been possible

Abstract

With the use of a digital ultrasound recorder, vocalizations were recorded from rats subjected to intense exercise conditions. The stress responsiveness of the animals was then quantified in terms of the high frequency vocalizations emitted by the rats. A newly developed clustering procedure was subsequently applied to the vocalization data. The procedure identified two distinct stress call patterns which reflected the intensity with which the rats were affected by the training regime. In addition, the recorded vocalizations suggested that the males reacted much more intensely to the stressors than their female counterparts.

Acknowledgements

It is a pleasure to acknowledge the contribution of various individuals to the production of this thesis. I would like to thank professors Angelo Belcastro and David Secord for their helpful insight in outlining the research goals, and in addition for providing the means to actually carry out the research. I would also like to thank fellow graduate students Rupe Jain and Eric Fisher for the many enlightening discussion sessions that we embarked upon. I would particularly like to acknowledge the invaluable assistance of Fred Schindler, whose spirit truely exemplifies the meaning of the word *friend*. Finally, I would like to acknowledge my appreciation to Dr. W.A.G. Voss, to whom I am greatly indebted. It is through his efforts that this is all possible.

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I. Introduction

Over the last several decades, the prominance of the term 'stress' has dramatically increased. However, at the same time, the actual definition of stress has become successively blurred. Stated quite simply, stress deals with adaptability. If an organism is unable to adapt to certain stimuli, then it may enter what is termed the 'state of stress'. The state of stress is associated with numerous physiological and psychological changes and is believed to facilitate the disease process. In fact, it is estimated that up to 90% of all disease is stress related [Bowers et al.,1980]. A convenient means of studying incumbant stress levels is to employ model systems involving laboratory rats.

Rats have been extensively utilized in many realms of research. However, physiological and biochemical parameters are usually employed to assess their stress responsiveness. "A procedure frequently adopted for both young and adult rats involves the decapitation of the animals after exposure to a stress agent for analysis of corticosterone and ascorbic acid levels"[Nitschke, 1982]. Over the last decade, researchers have been evaluating the adequacy of employing rat vocalizations as a measure of the stress response. However, the majority of their work has been limited to the analysis of vocalizations elicited by cold and pain stress. This brings us to the overall objectives of the thesis:

1. To record rodent vocalizations elicited in response to exercise conditions
2. To comprehensively analyze the vocalization parameters

Due to the large number of vocalization parameters, multivariate analysis techniques were required to analyze the data. Two distinctly different techniques were employed:

1. hierarchical log-linear analysis
2. cluster analysis

Hierarchical log-linear analysis techniques attempt to fit models to multivariate, ordinal data. These techniques were employed only to a limited extent in order to assess the contributions of the various vocalization parameters to the model. Cluster analysis techniques attempt to reduce an original set of correlated variables to a smaller set that will preserve most of the original information. This can be accomplished by grouping the objects into clusters such that the elements within a cluster are more like each other than they are to elements in other clusters. However, there is an inherent liability associated with standard clustering methods in that for large numbers of objects, the costs become very prohibitive. Thus, a new clustering routine entitled *CLUSTREE* was created based on an algorithm initially developed by Zupan[1982].

The thesis can be broken down into four parts:

1. An Introduction to Stress Research :
2. Rat Communication
3. Cluster Analysis Algorithms
4. Analysis of Vocalizations

The first three parts serve primarily introductory roles; the fourth part overviews the methodology required to record the vocalizations and then discusses the results obtained from the recording sessions.

II. Stress Research

A. History

Research in the stress field began long before the term 'stress' figured prominantly in the medical field. Claude Bernard, a French physiologist in the mid nineteenth century, pioneered stress research by introducing the concept of an internal environment. He stated that each cell of a complex multicellular organism is bathed by fluid called extracellular fluid which constitutes the immediate environment of the cells. This extracellular fluid serves as the medium of exchange with the cell for oxygen, carbon dioxide, waste and numerous other substances. Bernard considered that all physiologic mechanisms have one adaptive consequence, namely that of preserving the conditions of the internal environment, or as Bernard labeled it, ***le milieu interne***.

In actuality, the extracellular fluid can be redefined on two levels:

- 20% is confined within blood and lymph vessels and constitutes the *plasma*
- 80% is directly dispersed around and among the cells and is known as *interstitial fluid*

Fluid is also present inside the cells. This internal fluid is called the intracellular fluid and is separated from the extracellular fluid by the cell membrane.

The concept of regulating the internal environment was further developed by an American physiologist named Walter Cannon. Cannon postulated that tissues and organs must be regulated and integrated with each other in such a way that any change occuring in the internal environment initiated a counteracting reaction to minimize the change. He defined this maintenance of steady state within the tissues *homeostasis.* Changes in the internal environment are mediated from changes in the external environment via the dynamic component of extracellular fluid – blood circulation. This component responds both physically and chemically to changes in the external environment. In turn, the composition of the interstitial fluid changes since it is dependant on the conditions of the circulating blood. The net effect results in a change in the internal environment of the cell.

Cannon also postulated the *Emergency Theory*, commonly referred to as the *fight or flight* response, which describes the general function of epinephrine secretion from the adrenal medullae.

The term 'stress' was first introduced to the health sciences by Hans Selye[1950]. Selye's research was initiated by noting that individuals suffering from a wide range of physical ailments all seemed to have a group of similar ailments. Selye went on to call this condition the "syndrome of just being sick".

1. Formulation of the General Adaptation Syndrome

In his early research, Selye noted that animals exposed to a variety of non-specific damaging agents responded with a discharge of epinephrine and adrenal cortical hormones. He was the first person to identify the role of the adrenal cortex in the stress response. Of primary significance to Selye was the fact that the response was non-specific in that any stress was met by an increased discharge of corticoids. This suggested to him that perhaps all the systemic diseases are met by similar defensive corticoid production. His next attempt was to determine how the adrenal cortex was activated.

Selye noted that if an animal's pituitary gland was removed, it could no longer respond with any adrenal-cortical secretion. This was observed even when the animal was exposed to severe stress levels. Under these conditions, he also noted that certain illnesses (such as gastric ulcers) intensified while most of the presumably defensive changes were eliminated. This suggested to him that organisms may have inherent general defense mechanisms in which cortisol (a hormone secreted by the adrenal cortex) production plays an important role. Selye also saw a promising new way in which to treat stress related diseases - namely by administrating supplements of corticoids or ACTH when required. Several years later however, it became evident that increased bodily production of cortisol during stress or external administration of ACTH and corticoids can in itself become the cause of the disease. For example, Selye named hypertension, arterio sclerosis and gout as diseases that occured primarily due to excessive cortical levels. All of these findings led Selye to formulate the *GENERAL ADAPTATION SYNDROME (G-A-S)*.

a. The General Adaptation Syndrome

The basis of the general adaptation syndrome is that the response of the body to stress is largely dependant upon the function of the nervous system. The G–A–S is not simply a transitory adjustment to change, it is an adaptive reaction. It includes the learning of defense against future exposure to stress, and helps to maintain a state of adaptation once defence is acquired.

The G–A–S develops in three stages:

The Alarm Reaction

This stage represents the effects of non–specific stimuli on large portions of the body and can be divided into two distinct phases:

The Phase of Shock. The phase of shock is characterized by a number of symptoms including hypothermia, hypertension, decrease in muscular tone and genearalized tissue breakdown. There is also a discharge of epinephrine, ACTH, and corticoids which serve as early defense mechanisms.

The Phase of Counter–shock. The phase of countershock represents the system of defense against shock and is characterized by a reversal of most of the changes seen during the shock phase.

Stage of Resistance

The stage of resistance represents the systemic reactions elicited by exposure to stimuli in which the organism has acquired adaptation. It functions in such a manner that increased resistance to a particular stressor will effectly decrease the resistance to the other stressor(s).

Stage of Exhaustion

The stage of exhaustion represents the systemic reactions which develop due to chronic exposure to stimuli. In such cases, adaptation was developed but could not be maintained. Even perfectly adapted organisms cannot maintain themselves indefinitely in a resistance stage.

B. A Definition of Stress

Over the past few decades, literally tens of thousands of articles about stress have been written, but there is little coherence in the theory and research documented in these articles. The problem with research in the field of stress is that there exists no unilateral conventions concerning terminology associated with stress.

Originally, the term 'stress' was used to define the state of human difficulty caused by external factors beyond ones' control. A number of new definitions of stress have been recently formulated. Stress is considered by some to be a *state of mind* in which anxiety determines an individual's behavior. Other researchers substitute the term conflict or frustation in place of anxiety in the above definition. Stress is also regarded as *anything that causes alteration of homeostatic processes.* Many researchers consider stress as *any demands that evoke adaptive responses.* The large number of conflicting definitions of stress illuminate the need for a more precise definition and theory of stress.

1. The Stress-Disease Relationship

Stress is studied because of its implication in many disease processes. Even though some researchers (Kenneth et al.,1980) have stated that the correlation between stress and disease can be as low as 0.10, the majority of researhers strongly believe that stress figures prominantly in the disease process. These researchers believe that tissue systems may function in a less integrated manner under stress conditions. Cardiovascular disease, hypertension, mental illness and gastrointestinal disorders are examples of diseases that are thought to be facilitated to some degree by the presence of stress.

a. The Disease Process

Control systems seldomly operate precisely at their established levels of equilibrium. Instead, they tend to slightly oscillate about these operating points. As the degree of stress experienced by the organism increases, the oscillation levels increase coincidingly. If the oscillations exceed a certain threshold level, then effective stability control is forfieted. This may in turn lead to cellular damage which is characterized as *disease* [Ramsey,1982]. Control system failure may also occur if the control system is forced to operate too hard for extended periods of time (eg. intense exercise).

2. Stressor Types

Three types of stressors are known to exist: social, psychological and physiological. Technological advances have greatly reduced the number of physical stressors present in our environment. However, the advances that have freed man from the most extreme types of physical stressors have at the same time exposed him to ever increasing levels of psychological (or social) stress. Our society is constantly changing and these social changes are eliciting psychological stress. Many researchers believe that psychological stress levels are reaching alarming proportions. It has been noted [Ramsey, 1981] that rapid social changes are associated with elevated risks of illneses such as diabetes and leukemia, as well as the increased occurence of a wide assortment of minor complaints.

Physiological Stress. Traditionally, physiologic stress was the first realm of stress to be studied. Research by Selye triggered a *snowball* effect that saw the interest in physiologic stress greatly increase. Today, physiologic stress is studied mainly to establish the correlation between stress and the incidence of disease. "Practical considerations of physiologic stress inevitably center around its potential relationship to disease. Do stressors produce disease? ... Stress begins to manifest itself as a disease process when homeostatic mecahnisms become faulty and can not cope with continued stress." [Ramsey, 1981]. The distinction between physiologic stress and disease is contingent upon two factors:

- the degree and duration of the stressor
- the effectiveness of the counteracting adaptive response of the organism

In reality, we are seldomly confronted with only one stressor during any given time. It is known that the response to a particular stressor can automatically alter the responses to other simultaneous stressors. This usually results in a less effective response to any one stressor, and hence a less effective overall response. Individuals face a strong risk of developing disease during conditions of chronic exposure to multiple stressors.

Psychological Stress. Many researhers believe that in order to preserve psychological homeostasis, individuals must be able to maintain a *normal* mood state. Thus emotions, which are deviations from this normal mood state, reflect the condition of psychological stress. Others believe that the variable of *threat* distinguishes psychological stress from the other types. Despite the large number of theories concerning the definition of psychological stress, most researhers agree that cognitive informantion processes figure prominantly in determining the responses to psychological stressors.

> The key feature of psychological stress that distinguishes it from stress at the social and physiological levels is the presumption that cognitive activities – evaluative perceptions, thoughts and inferences – are used by the person to interpret and guide every adaptational interchange with the environment. The person is said to appraise each ongoing and changing transaction with the environment with respect to its significance for the person's well being. This appraisal includes judgements (whether conscious or unconscious) about environmental demands and constraints as well as about the persons resources and options for managing them. (Lazarus 1971)

It is interesting to note that psychological stress processes result in physiological reactions identical to those directly produced by physiologic stimuli. This tends to indicate that both forms of stress may share some common pathway in the stress response. Also, a large amount of the physiologic stress that individuals must endure is mentally provoked. In our abilities to anticipate and perceive stress, we tend to complicate the stress response with the addition of psychological stress in the form of *dread*. Thus, in order to lessen the effects of stress, we must learn to direct our thoughts away from the impending stressors.

Social Stress. Social stress can be viewed as *psychological reactions prompted by social interactions*. Thus the term psychosocial stress is commonly used in conjunction with social stress. In today's society, social stress presents itself as an ever-increasing stigma. This is reflected by the high incidence of crime, mental illness, divorce and smoking that is present in our society. Ramsey[1981] defines psychosocial stressors as "stimuli originating from interpersonnal interactions and social arrangements whose initial effects are mediated through the senses and the higher neural processes." Thus, social stress reactions are very similar to psychological stress reactions. However, social stress tends to reinforce itself in a positive feedback system. The psychological and physiological changes elicited by social stress tend to intensify the existing physiological changes and may even initiate new responses.

C. The Nature of Stress Reactions

In reviewing the stress literature, researchers have been able to differentiate the reponses to stress into four categories:

- disturbing affects such as fear, anger and anxiety
- motor behaviors such as facial expression and muscle tension
- changes in cognitive functioning which affect perception, judgement and threat
- physiological changes integrated by the neural and endocrine systems

A rigorous description of the physiological changes is given in a following section.

D. Systems of the Stress Response

In order to maintain homeostasis, unmitigated control of the regulatory functions is essential. The neural and endocrine systems represent the primary control systems in the body. The nervous system provides rapid response and coordination of the internal organ systems. The endocrine system lacks the immediate response of the neural system, however its effects are much longer in duration. Both systems play significant roles in the maintenance of homeostasis. The combination of both systems, referred to as the neuroendocrine system, forms an intricate regulatory system that figures prominantly in the stress response.

E. The Stress Response

The stress response can be evoked in two ways, externally and internally. The external pathway involves the perception of potentially harmful stimuli by sensory receptors located throughout the body. Once stimulated, the receptors send impulses to the brain via the peripheral nervous system. Conversely, the internal pathway responds to stressful stimuli that have been internally initiated by processes such as imagination and recollection. They differ from the external pathways in that the incoming functions of the peripheral nervous system are bypassed.

Once the impulses reach the brain, an interpretive analysis is made. This interpretive analysis involves two main areas, the higher levels of the brain and the limbic system, working in conjunction. If the stimulus is interpreted as being a threat or challenge, emotional arousal will most likely result. Snyder[1974] summarizes this as "events preceived in the environment may be integrated with ... emotional states encoded in the hypothalamus and the limbic system". Thus the stress response results from processes of cognitive interpretation and emotional arousal and not from the stimulus itself. It is for this reason that the same stimuli can elicit a variety of effects in different individuals. The stress response can be thought of in terms of a process with four constituent parts or *axes* [Everly & Rosenfeld, 1983] and is depicted in figure 2-1.

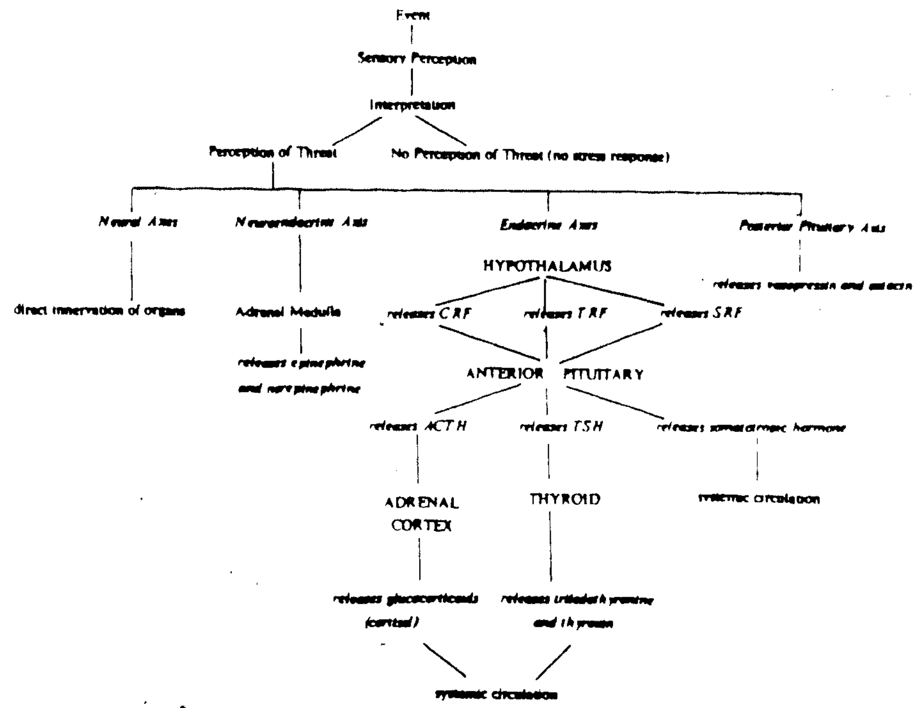


Figure 2-1 The Stress Response

All of the axes of the stress response begin with the delivery of information concerning the potentially stressful stimuli to the hypothalamus. The hypothalamus serves as a central integrating area for the stress response process. It is involved in integrating both neural and endocrine responses to stress and is in turn regulated by both the nervous system and the levels of circulating hormones. Thus the hypothalamus acts as a purely nervous center and also as a neurosectory organ.

Each of the stress response axes corresponds with one of the four known ways in which the hypothalamus responds to a stressful situation:

1. *Neural Axes* – causes the stimulation of the autonomic nervous system

2. *Neuroendocrine Axis* – causes the adrenal medullae to secrete epinephrine into the systemic circulation

3. *Endocrine Axes* – secretes the releasing hormones CRH, SRF, and TRH into the hypothalmic–hypophyseal portal system

4. *Posterior Pituitary Axis* – causes the release of antidiuretic hormone and oxytocin into the systemic circulation

1. The Neural Axes

The neural axes represent the level of response that occurs through the direct innervation of target organs. Two neural axes exist, namely the sympathetic and parasympathetic branches of the autonomic nervous sytem. Sympathetic activity is usually found to be dominant and results in the release of norepinephrine to the various target organs . However, parasympathetic activity is also elicited and can become dominant in some forms of stress.

Since these pathways are neural in nature, they respond very rapidly. For this reason, the neural axes are the first axes of the stress response to be activated. Unfortunately, their results are short–lived. This is due to the limited ability of the autonomic nervous system to continually release neurotransmitter substances. Thus the neural axes are characterized by a rapid reponse that is short in duration.

Activation of the neural axes results in increased blood pressure, increased cardiac output, increased respiration rate, decreased rate of blood flow to the viscera and increased rate of blood flow to the muscles.

2. The Neuroendocrine Axis

The neuroendocrine axis was first identified by Walter Cannon, who labeled it the *fight or flight response* . Its systemic effects are centered around the role of the adrenal medullae. Adrenal medullary stimulation elicits the secretion of the catecholamine hormones epinephrine and norepinephrine which produce virtually the same effects as direct sympathetic innervation. Unlike the neural axes, the neuroendocrine axis is associated with an activation delay time on the order of twenty to thirty seconds. However once it begins to act, the neuroendocrine axis displays a response that usually lasts at least ten times longer then the neural response. Secretions from the adrenal medullae act to prolong and reinforce the direct sympathetic effects.

The response of the neuroendocrine axis depends upon the level of stimulation. As expected, strong levels of stimulation elicit high levels of response; however, levels of stimulation below a nominal value can also elicit high levels of response [Frankenhauser, 1980] as shown in figure 2-2. The two conditions that correspond to these high response levels are called overload and underload. Both conditions are similar in that they are deviations from the level of stimulation the person is normally accustomed to. The neuroendocrine axis is active whenever either of these conditions exist.

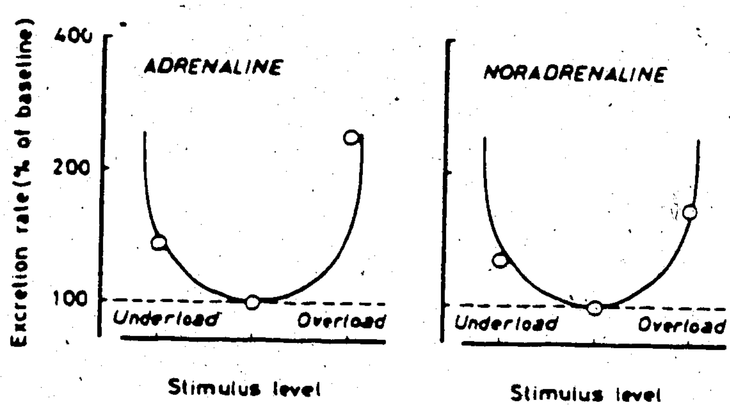


Figure 2-2 The Relationship Between Stimulation Levels and Stress

It is important to note that in situations of repeated exposure, only the norepinephrine level is always significantly elevated. Thus the epinephrine level shows a tendency to decrease with repeated exposure. However, in situations were a high degree of envolvement must be maintained, epinephrine levels remain significantly elevated

3. The Endocrine Axes

At the present time , three endocrine axes are known to exist. These include the adrenal cortical axis, the somatotropic axis and the thyroid axis. Intense stress levels must exist before these axes will be activated. Once activated, their effects remain for relatively long periods of time.

i) Adrenal Cortical Axis. Pioneer work by Selye showed that the activity of the adrenal cortex was a major constituent of the stress response. The adrenal cortical axis involves a series of feedback loops which control the levels of various circulating hormones.

As with all the response axes, the adrenal cortical axes begins with the transmission of information to the hypothalamus. Once stimulated, the hypothalamus secretes a hormone called corticotropin releasing factor (CRF) which stimulates the anterior pituitary to secrete excess amounts of the hormone adrenocorticotropin (ACTH) into the systemic circulation. In the presence of stressful stimuli, ACTH secretion can be increased up to levels that are twenty times the resting level within a few minutes. The ACTH levels in turn stimulate the adrenal cortex to increase its synthesis of hormones, particularly the glucocorticoids. Thus increases in ACTH levels always precede the elevation of the glucocorticoid levels. About 95% of the glucocorticoid activity is due to cortisol, also known as hydrocortisone. The primary effects of cortisol are the regulation of blood pressure and the enhancement of blood glucose levels.

In situations were stressors are continually present (such as mental strain, thermal change, excessive noise and inadequate rest), the adrenal reserve of glucocorticoids may become temporarily depleted. In this situation, the effects of the adrenal cortical axis become very limited.

Feedback Loops in the Adrenal Cortical Response

Control of the adrenal cortical axes is maintained through the use of negative feedback. Three types of feedback systems are known to exist.

1) Long Feedback Loop. The effects of cortisol act in some way upon the hypothalamus to inhibit the secretion of CRH. This in turn inhibits the secretion of ACTH and cortisol, thus restoring the hormonal balance.

2) Short Feedback Loops. Two short feedback loops exist. ACTH acts upon the hypothalmic nuclei to inhibit CRH secretion, forming one of these feedback loops. The other loop is formed by the actions of cortisol upon the anterior pituitary which inhibit the secretion of ACTH.

3) Ultrashort Feedback Loop. Recent evidence [Schmidt & Thews, 1983] suggests that high levels of CRH act directly upon the hypothalmic nuclei to decrease the secretion of CRH.

The feedback systems may be thought of as having *set points* which determine the levels at which negative feedback begin. In situations of continual high levels of stress, ACTH secretion may continue irregardless of existing cortisol levels. Thus the set points should be considered as *floating* in that they can fluctuate according to residing stress levels. Conditions which raise the set points to abnormally high levels can lead to loss of control of the feedback system. Under such conditions, increased susceptibility to disease is exhibited [Ramsey, 1982].

ii) The Somatotropic Axis. The somatotropic axis shares part of its pathway with the adrenal cortical axis. It responds to stress by releasing somatotropic releasing factor (SRF) from the hypothalamus. The SRF in turn stimulates the anterior pituitary to release somatotropic hormone (STH), also known as growth hormone, into the systemic circulation.

Exertion, exposure to cold, trauma and emotional arousal are stressors known to elevate STH levels.

iii) The Thyroid Axis. The role of the thyroid axis in the stress response is obscure. It is known that it can be activated by psychological stimulation.

The thyroid axis begins with the hypothalmic release of thyrotropin releasing factor (TRF) into the hypothalmic – hypophyseal portal system. Once TRF reaches the anterior pituitary, the anterior pituitary is stimulated to secrete thyroid releasing hormone (TSH) into the systemic circulation. TSH in turn stimulates the thyroid gland to release the hormones triiodothyronine (T3) and thyroxin (T4) into the circulation.

Some stressors, however, act to diminish the output of thyroid hormones. Also, studies (Ramsey, 1982) have led to the conclusion that there may be an inverse relationship between thyroid output and cortical activity.

4. The Posterior Pituitary Axis

Stimulation of the hypothalmic nuclei can result in the secretion of antidiuretic hormone (ADH) and oxytocin into the circulation. It is speculated that these hormones do not play a major role in the stress response, and hence the existence of a pituitary axis in the stress response is questionable. It is known that ADH acts to promote the retention of water.

III. Rodent Communication

It has only been over the past few decades that technical developments have permitted us to explore the realm of *ultrasonic* communication between animals. Ultrasound, which encompasses all sounds with frequencies above 20 kHz., is the media that many animals, including rats, use to communicate with each other.

It was originally thought that laboratory rats were relatively silent creatures. "If after weaning, a rat is put into an individual cage, it can be counted on, if not disturbed, to live the rest of its life and die without a single vocalization"[Mowrer et al., 1948]. The first report of purely ultrasonic vocalizations in rats was made in 1954 by Anderson when he detected isolation calls from adult laboratory rats (*Rattus Norvegicus*) at frequencies of 23 – 28 kHz. and durations of 1 – 2 s. Anderson speculated that these calls may facilitate either communication between individuals or echo–location.

To date, researchers have studied numerous conditions under which rats emit ultrasound. Table 3–1 summarizes some of these conditions.

TABLE 3-1 RAT VOCALIZATION CONDITIONS

Age	Frequency (kHz.)	Duration (ms.)	Comments
infants	40-95	5-65	isolation from nest, calls figure prominantly in the mother-young relationship
adult males	40-50	100-500	calls elicited during mounting and intromission
adults	40-50		short pulses indicate aggresion whereas long pulses indicate submission
adult males	22-25	2000-3000	pre-ejaculatory/ post-ejaculatory signal
adult females	not given	short pulses	males entering cage
adult females	22-30	not given	calls have been recorded from lactating females whose litters have been removed
adult males	22-30	700-1000	calls indicate the sexual motivation of the male and tend to inhibit aggresion by the female

Many of the rodent vocalizations that researchers have recorded fall within two distinct frequency ranges: 20 – 28 kHz. and 40 – 50 kHz. The 40 – 50 kHZ. calls are primarily emitted by the infant rats, whereas the lower frequency calls are characteristic of adult vocalizations.

A. Models for the Elicitation of Stress Vocalizations

Researchers have formulated three different models that describe the elicitation of stress vocalizations. Each of these models attends that an arousal process is at least partially responsible for the vocalizations.

1. Arousal Model

Arousal models attribute behavior to levels of arousal. The state of arousal depends upon two factors: internal states of bodily imbalance and external stimulus conditions. Changes in the external stimulus field can either increase or decrease the level of arousal.

The first individual to link ultrasonic signaling with arousal was Bell[1974]. However, Bell states that vocalizations should not be labeled with their correlated behavior in such a manner that a signal-behavior list is compiled. Instead, he suggests that the vocalizations represent the general state of arousal of the rodent.

2. Neuronal Model

The neuronal model can be considerred as an extension of the arousal model in that it includes the developmental process along with the arousal process. In his reformulation of the original neuronal model[Sokolov, 1960], Salzen[1970] states that "organisms fashion appropriate neurological models of the environment on the basis of perceived stimulations". Any discrepancies from established models result in arousal. If the state of arousal leads to a new stimulus source that matches the neurological model, then the arousal level is lowered, otherwise a new model is created. Mild discrepancies between incoming information and existing models produce adaptive behavior; large discrepancies produce disruptive behavior which may in turn elicit stress vocalizations.

3. Pro–Comp/Arousal Model

The pro–comp/arousal model was formulated by Nitschke[1982] as an alternative to existing models. It incorporates arousal only as the energizer of behavior. The consequences of the energized behavior can then in turn either lower or raise the level of arousal. The model also incorporates a behavioral process in which the internal and external environments are continuously monitered. Two systems have access to the monitered signals: the arousal system and a program comparator(pro–comp). The pro–comp first compares incoming information with a register of normative sensory values and then inputs the degree of concordance to the arousal system. As concordance levels decrease, arousal levels increase. "In the case of exceedingly high arousal, it can be assumed that the resulting behavior can be characterized as highly emotional and nonadaptive"[Nitschke, 1982]. Under such situations, ultrasonic vocalizations are emitted. The model is depicted in figure 3–1.

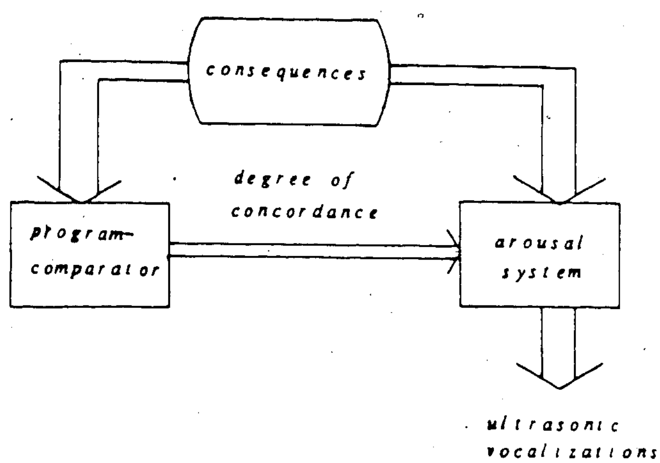


Figure 3–1 The pro–comp/arousal model for the elicitation of ultrasonic vocalizations

IV. Cluster Analysis

A. Similarity Measures

Most clustering techniques begin with the calculation of a matrix of similarities or distances between individuals. Similarity measures are usually viewed in relative terms. Two objects are said to be similar if they exhibit aspects in common, relative to those which other pairs of objects share. Measures of similarity generally fall into two categories: distance type measures and matching type measures.

1. Distance Type Measures

The most commonly used distance measure is the Euclidean distance function. This function denotes the distance between points *i* and *j* by *d*, given by

$$d(X_i, X_j) = \left[\sum_m (X_{im} - X_{jm})^2 \right]^{1/2} \qquad [1]$$

where *m* specifies the dimension of the space in which the points are located. However, this type of distance measure does have its drawbacks in that distance can be adversely affected by changing the scale of a variable. Therefore it is possible that techniques that use the Euclidean distance function will not preserve distance rankings if scale changes are brought about.

2. Matching Type Measures

Matching type measures are used when the data being analyzed are expressed as correlation coefficients, binary data illustrating a prominent form of data expressed in this manner. A simple matching measure can be stated as:

$$S = Ne / Nt \qquad [2]$$

where

Ne = number of attributes whose coefficients are equal for both objects i and j

Nt = total number of attributes

The matching measure need not be limited to the form given by equation [2] and can take on many different forms.

B. Cluster Analysis Techniques

Cluster analysis techniques seek to separate data sets into groups or clusters and may themselves be classified in the following manner:

1. *Hierarchical* techniques
2. *Optimization-Partitioning* techniques
3. *Density* or *mode-seeking* techniques, and
4. *Clumping* techniques.

1. Hierarchical Techniques

Hierarchical techniques may be further divided into agglomerative methods and devisive methods. Agglomerative methods proceed by the successive fusing of individuals into groups; devisive methods successively partition the set (initially comprised of all individuals) into smaller subsets. *Dendograms*, which are two-dimensional diagrams illustrating the fusions or partitions which have been made at each successive level, are used to illustrate the results of both techniques.

a. Agglomerative Methods

These methods begin with the computation of a similarity or distance matrix between the individuals. Individuals or groups of individuals are then fused together, based on their similarities or relative distances to each other.

There are several agglomerative hierarchical techniques presently used. They differ from each other in the sense of how they define distance or similarity between individuals in the data sets.

Single Linkage Method

Groups, initially consisting of single individuals, are fused according to the distance between their nearest members, the groups with the smallest distance being fused. Each fusion successively decreases the number of groups by one.

Complete Linkage Method

This method differs from the single linkage method in that the distance between groups is defined as the distance between their most remote pair of individuals.

Centroid Cluster Analysis

As the name implies, this method represents groups in the p-dimensional space by the coordinates of their centroids. The distance between groups is then defined as the distance between group centroids. Groups are fused acccording to the distance between centroids, with the groups with the smallest distance being fused first. This method has a disadvantage however, in that the characteristic properties of a smaller group are lost if the sizes of the two groups to be fused are greatly different from each other. In this case, the centroid of the new group will be within close proximity of that of the larger group and may remain within that group.

Median Cluster Analysis

This method overcomes the disadvantage of centroid cluster analysis by first assuming that the groups to be fused are of equal size. The position of the new group will then always be between the two groups to be fused.

Group Average Method

The group average method takes into account all individuals within the groups by defining distance between two groups as the average of the distance between all pairs of individuals in the two groups.

Wards Method

At any stage of an analysis loss of information results from the grouping of individuals into clusters. Wards method is based upon the minimization of a particular measure of loss. This measure is given by the total sum of the squared deviations of every point from the mean of the cluster to which it belongs. Thus the choice of the two clusters to be fused will result in a minimum increase in error.

b. Devisive Methods

Devisive methods are initiated by fragmenting the set of individuals into two. Each set of *n* individuals can then be further divided into two subsets, with the process being repeated until a set limit is reached. There are two basic families of devise techniques: monothetic, based on the posession of a single specified attribute, and polythetic, based on the values taken by all the attributes. Monothetic techniques are usually used in cases where binary data are present and will not be discussed further. Polythetic techniques begin by choosing an individual to initiate a splinter group. This individual is chosen in a manner such that the average distance involved from the remaining individuals is a maximum. Next, the average distance to each individual in the main group to the individual in the splinter group is found, followed by the average distance of each individual in the main group to the remaining individuals in this group. For positive differences of the average distance from the splinter group from the average distance from the main group, the individual is added to the splinter group. If all of the differences are negative, the process is halted, but can resume on each of the two sub groups.

2. Partitioning Techniques

Partitioning techniques differ from hierarchical techniques in that relocation of the individuals is permitted to correct poor for initial partitions. The majority of these techniques can be summed up by three steps:

1. initiating clusters
2. allocating individuals to initiated clusters
3. reallocating some or all of the individuals to other clusters once the initial categorization process has been completed.

a. Cluster Initialization Techniques

These techniques commence by finding k points in the p-dimensional space, where k specifies the number of clusters to be formed and is predefined. These points act as initial estimates of the cluster center. Various methods have been proposed for choosing these points. One method looks at all of the data vectors and chooses the initial k mean vectors. Other methods choose the k points mutually furthest apart.

b. Relocation Techniques

Relocation techniques attempt to optimize a specified clustering criterion. Each individual is considered for reassignment to another cluster with reassignment actually taking place if it causes the desired modification of the criterion value. The procedure is then complete when no further move of a single individual causes an improvement.

3. Density Search Techniques

The concept of clustering suggests that there should be two types of regions in the p-dimensional space: regions in which the points are extremely dense separted by regions of low density. Density search techniques seek to find these regions of high density and usually incorporate the methods used in single linkage cluster analysis.

a. Mode Analysis

Mode analysis, a derivative of single linkage clustering, searches for natural sub-groupings of the data by considering a sphere of some fixed radius R surrounding each point. Those individuals whose spheres contain a large number of points in relation to the total number of points are called dense points (the minimun number of points required for an individual to be labeled as dense is given by k). The radius R is then gradually increased, allowing more inidividuals to become consolidated. Four courses of action become possible with the introduction of each new dense point:

- The new point is separated from all other dense points by a distance which exceeds R. This initiates a new cluster nucleus and hence the number of clusters is increased by one.

- The new point is within R units of one or more dense points which belong to only one cluster nucleus. In this case, the new point joins that cluster.

- The new point is within R units of dense points belonging to two or more clusters, in which case the clusters concerned are combined.

- At the introduction of each new cycle in which R is increased, the smallest distance D between dense points belonging to different clusters is found. This distance is compared with a threshold value computed from the average of the $2k$ smallest distance coefficients for each individual. If D is less than this threshold value, then the two clusters are combined.

4. Clumping Techniques

Clumping techniques differ from other methods in that they permit the overlapping of clusters. They begin with the computation of a similarity matrix and then seek a partition of the individuals into two groups.

Clumping techniques are frequently employed in fields such as language studies where classification must permit the overlapping of classes, thus acknowledging the existence of multiple interpretations for many words.

C. Cluster Discription and Significance

Once clusters have been developed, the task of describing the clusters still remains to be resolved. One measure frequently used to describe clusters is based on the location of the cluster, which can be represented in a number of different ways. The centroid of the cluster, given by the average value of the objects contained in the cluster on each of the variables making up their profiles, is one way. In addition some measure of the cluster's variability may be computed – for example, the average distance between all pairs of points within the cluster.

The number of different types of measures which can be used to describe the various aspects of a cluster is immense. Thus, the original purpose of the analysis must be considered as it may limit the number of ways in which the cluster can be simply described.

V. The Problem With Standard Clustering Methods

All standard clustering procedures follow a fundamental algorithm involving the continual updating of some form of distance or similarity matrix. "The distance matrix is the source of all troubles when standard clustering techniques are applied for generation of the hierarchical trees on large numbers of objects ... the real problem is that the distance matrix has to be used in a recursive manner in such a way that the number of accesses to its elements is proportional to the square of the number of elements" [Zupan,1983]. Thus, for large numbers of objects, the costs associated with standard clustering methods become very prohibitive. A new method has been developed by Zupan which overcomes the cost deficiency of standard clustering methods. Central to the methodology of this new clustering technique is the use of a comparison tree. This tree is used for the generation of the large hierarchical tree found in almost all clustering schemes.

A. Comparison Trees

A comparison tree is composed of the following elements: a *root*, which denotes the top of the tree; *vertices*, which are branching points; *branches*, which connect various vertices with other vertices; and *leaves*, which are end vertices that have no further branches. Other terminology frequently associated with trees includes the phrases *children*, *parent*, and *external and internal path lengths*. Parent and children refer to the vertices immediately above and below a particular vertex, respectively. External path length refers to the sum of the number of branches traversed in going from the root to every leaf in the tree; internal path length refers to the sum of the number of branches in going from the root to every *non-leaf* vertex.

The new clustering method uses a particular type of comparison tree called a *2-tree* . A 2-tree, also known as a *strictly binary* or an *extended binary tree* , is a tree in which every vertex except the leaves has exactly two children. Thus we have the following facts:

LEMMA 1. The number of vertices on each level of a 2-tree is at most twice the number on the level immediately above.

LEMMA 2. In a 2-tree, the number of vertices on level x is at most 2^x for t>0.

LEMMA 3. The total number of vertices in a 2-tree with n objects is always 2n-1.

The maximum number of edges between the root and the leaves of a tree is called the ***height*** of the tree. For a 2-tree containing n objects, it can be shown that the minimum height for the tree is given by:

$$Hmin = \lceil \log_2(n) \rceil \quad [3]$$

where $\lceil k \rceil$ is called the ceiling of k and is defined as the smallest integer greater than or equal to k. When the height of a particular tree is equal to minimum height, the tree is referred to as a *balanced* tree. The maximum height possible for a 2-tree is given by:

$$Hmax = n-1 \quad [4]$$

Trees with heights close to the maximum value are referred to as *heavily chained.* The term 'height' should not be confused with the term 'distance'; the height between two vertices is denoted by the number of edges between the two vertices whereas the distance between two vertices requires the use of a *Metric*, which employs a distance measure.

Comparison trees can serve two different purposes:

i) They can be used for retrieval purposes in which case the allocation of the objects is not important. For example, many assemblers and compilers use trees as symbol tables to keep track of user-defined symbols.

ii) They can be used for grouping purposes in which case object allocation is extremely important.

For the new clustering technique, correct object allocation is extremely important, hence trees will be used in terms of their grouping capabilities.

Up to this point, only binary trees have been mentioned. However, there may be applications for which the number of children of a given node should be arbitrary. Such trees are termed *ordered* trees. Fortunately, ordered trees can be converted into binary trees through the use of a rotation sequence[Knuth,1976]. Thus, what at first seemed to be a severe restriction in that each vertex could only contain a maximum of two children turns out to be of no major conseqeunce. In summary, binary trees provide a conveniant form of representation for a much larger class of ordered trees.

B. Discussion of the New Clustering Method

Before proceeding any further, some sort of convention must be set up as to how the clusters should be represented in the measurement space. Many elaborate cluster representations exist, however they tend to be very cost intensive. Since one of the primary concerns of the algorithm is efficiency, a simple cluster representation is required. For this reason, clusters are represented in terms of average vectors V of the n objects belonging to them. It is in this section of the clustering algorithm that the dimension of the measurement space greatly influences computation time; as the dimension increases to a value over 50, the costs rapidly become prohibitive.

There are situations for which the average vector representation is particularly bad (figure 5-1). If the data are organized in a manner as illustrated in figure 5-1, an alternate cluster representation must be chosen.

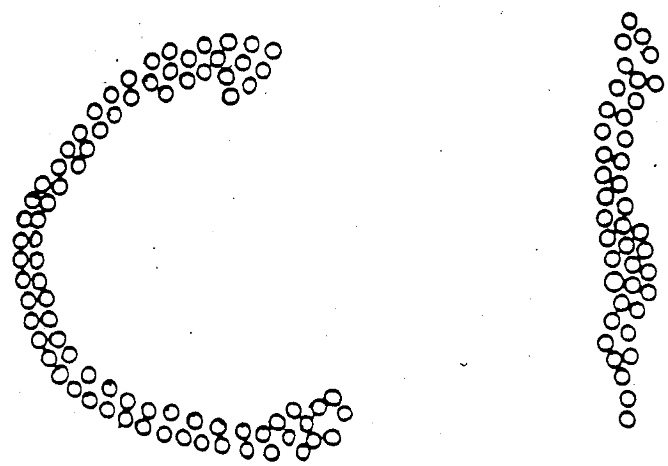


Figure 5-1 Situations for Which the Average Vector Method Should not be Employed

New objects are added to an existing cluster in the following manner:

$$V[n+1] = (V[n] \cdot n + X) / (n+1) \qquad [5]$$

This results in a vector that reflects the average of the n+1 objects in the cluster membership.

1. Two Distance Clustering Method

The first new clustering technique that will be discussed involves the use of two distances, namely the distances from a parent vertex to its left and right children. It begins with the initialization of the comparison tree. This is accomplished by creating a root and setting the first two data vectors to the left and right children of this root. New objects must always enter the tree via the root. Further traversal of an object through the tree is determined by an evaluation of the distances between the object and the left and right vertices, labeled *dl* and *dr* respectively; the minimum distance determines the path. Should the two distances be equal, the algorithm arbitrarily specifies a direction. The traversal is complete when the object reaches a leaf. At this point, two new vertices must be created; one to serve as a leaf that contains the new object and the other to serve as a *parent* for the old and new leafs. Thus, the tree is rearranged such that the new parent vertex occupies the previous position of the 'old' leaf. Figure 5–2 illustrates the process of adding a new object to a tree. The above procedures are repeated until all objects have been added to the tree.

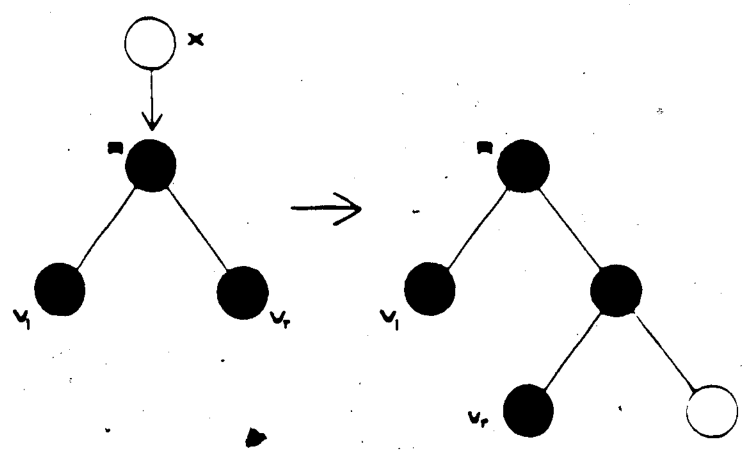


Figure 5–2 Addition of an Object into the Tree

Once initial object allocation is complete, the tree is 'tested' for its retrieval capability. If all objects can be successfully retrieved, then the clustering procedure is complete; if some objects cannot be retrieved, then these objects are removed from the existing tree structure. The objects that have been removed must then be reintroduced into the remaining tree structure. This process is called *updating* the hierarchical tree and is continued until either all objects can be successfully retrieved or the maximum number of updating iterations is reached.

The construction of a hierarchical tree in such a manner can be vastly superior, in terms of computational effort, to standard clustering methods. For a completely branched tree, the number of distance calculations executed by the routine is given by the following relationship:

$$\text{calc}[n] = \text{calc}[n-1] + \lfloor \log_2(n-1) \rfloor \quad [6]$$

where n denotes the number of objects to be allocated. For heavily chained trees, on the other hand, the algorithm must perform significantly higher numbers of distance calculations than for their balanced counterparts. For a maximally chained tree, the number of distance calculations that must be executed by the routine is given by:

$$\text{calc}[n] = \sum_{j=3}^{n} 2 \cdot (j-2) \quad [7]$$

which is comparable to the number of distance calculations performed by standard clustering algorithms, given by

$$\text{calc}(n) = [n-1]^2 \quad [8]$$

Thus, it becomes evident that the two distance clustering method can fall short of its goal (computational efficiency) under circumstances in which heavily chained trees are produced.

One of the foremost researchers in the field of binary trees is Donald Knuth. Knuth's extensive study on computing and binary trees is documented in the monumentous work The Art Computer of Programming which consists of three volumes. The third volume, entitled *Sorting and Searching*, deals exclusively with techniques and problems associated with the implementation of trees on computer systems. In this third volume, Knuth states that binary trees tend to grow in a balanced manner. Due to this consideration, the two distance clustering method can achieve a significant cost savings. Figure 5-3 contains a plot of the number of distance calculations required for the various considerations previously described as a function of the number of objects to be allocated.

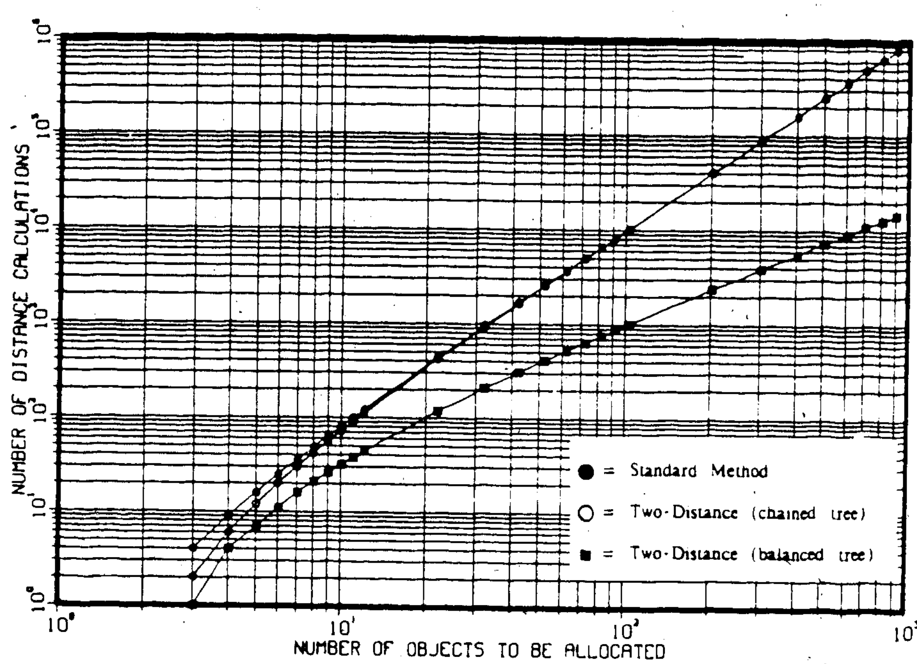
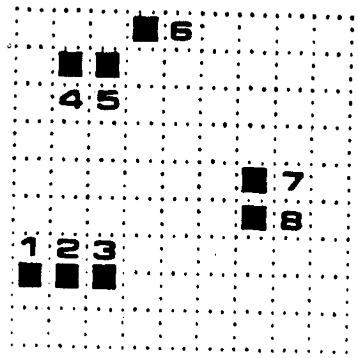
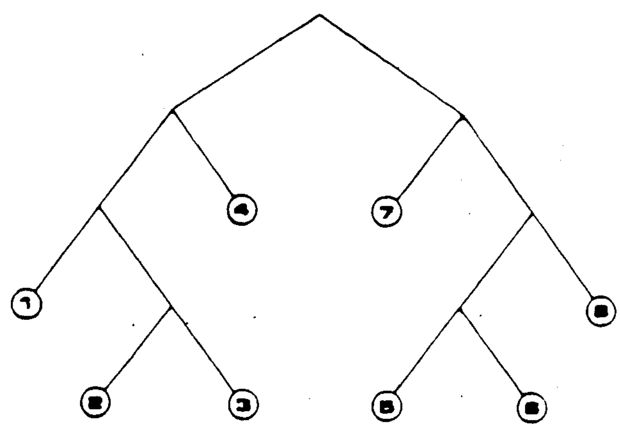


Figure 5–3 Cluster Algorithm Efficiencies

Unfortunately, there is an inherent liability associated with the two distance clustering method. It turns out that the characteristics of the tree are greatly affected by the order in which objects to be updated are presented. Thus, newly updated objects may be grouped into 'incorrect' clusters. Figure 5–4 shows an example of how input selection may result in an erroneous grouping of an object with a cluster distant from its measurement space.



a)



b)

Figure 5–4 The Influence of Input Selection on Object Allocation. a) original data points b) tree produced by the two distance method

2. Three Distance Clustering Method

The three distance clustering method evolved from the inadequacies of the two distance method. The idea of using a third distance was developed in terms of a solution to the following question:

What action should the clustering routine elicit when the object is 'far' from both the left and right vertices?

In other words, the two distance clustering method was deficient in that it did not take into account the actual position of the object compared to the positions of the left and right vertices. Thus, under no circumstances, was an object ever treated as an ***outliner***.

Before continuing any further, the concept of an outliner must first be quantified. An outliner is defined in terms of the distance between the left and right vertices of a particular vertex. This distance is given the label *d3*. Movement of an object through the tree is now contingent upon the evaluation of three distances:

$$dl = D(X,Vl) \quad [9]$$

$$dr = D(X,Vr) \quad [10]$$

$$d3 = D(Vl,Vr) \quad [11]$$

If the minimum of the three distances turns out to be d3, then the object is classified as an outliner and the tree must be disconnected; otherwise, the object traverses to the next level of the tree as dictated by the two distance method.

a. Disconnection of a Hierarchical Tree

The purpose of disconnecting a tree is to prevent the clustering of non-similar objects, which is a prevailent problem with the two distance clustering method. It requires the creation of two new vertices, Vx and Vy. One of these vertices, Vx, is placed between the vertex that the object is currently positioned at (V) and its parent (Vp); the other vertex, Vy, is positioned such that it is a child of Vx. Thus the tree is now structured such that Vx contains the new object along with all of the elements of V, and Vy contains only the new object. Figure 5-5 illustrates this process.

A situation may occur in which the tree is required to be broken at the root. In this context, the new object is viewed as an outliner to the entire cluster structure. Under such circumstances a new root must be created. This process is summarized in figure 5-6.

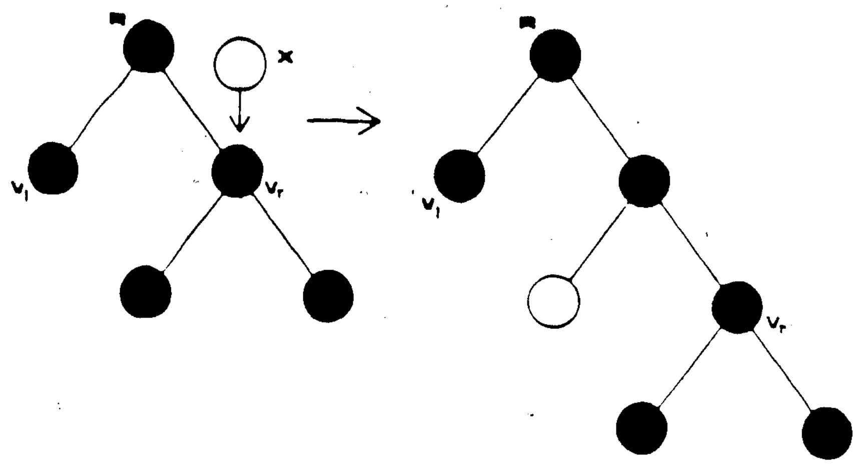


Figure 5-5 Disconnection of the Hierarchical Tree

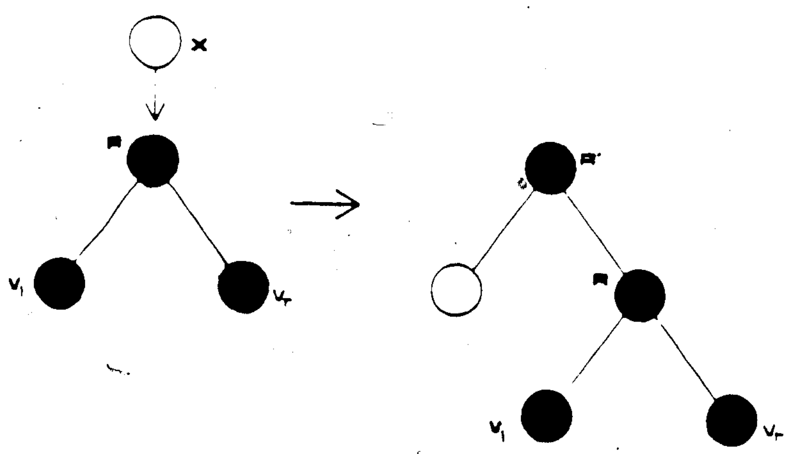


Figure 5-6 Formation of a New Root

The two-distance method allowed for the formation of leaves only at the end of the tree. With the addition of a third distance in the evaluation procedure, the three distance method permits leaves to be allocated throughout the tree structure. Thus the development of the tree is no longer rigidly circumscribed by the existing tree structure. However, the three distance method is still susceptible to errors during the updating sequence. To minimize these errors, a *safety wall* is introduced.

b. The Concept of a Safety Wall

"The most sensitive decision in the new algorithm is of course whether the vector X is an outliner or not, i.e., if the tree should be disconnected, new branch added, and the update terminated, or the traverse of the tree should be continued towards left or right descendant of the particular vertex" [Zupan, p 51]. The shaded region in figure 5–7 depicts the n–dimensional measurement space for which the tree is not broken: if an object lies within this space, then it will move either to Vl or Vr, depending on the minimum distance.

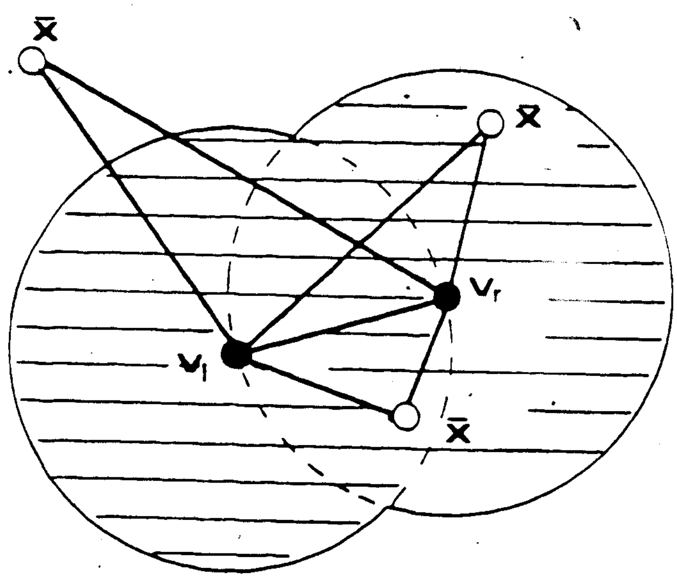


Figure 5–7 Illustration of the Three Distance Algorithm

The method works well for objects located within discrete, compact groups. However, for clusters that have objects widely dispersed from the cluster center, such as in figure 5–8, the validity of the clustering method comes into question. Under such conditions, "the clustering techniques tend to impose structure on the data rather than find the actual structure present"[Everitt, p. 44]. To lessen the severity of such clustering errors, a safety wall is introduced by altering d3 in the following manner:

$$d3' = d3 + dw \qquad [12]$$

where dw represents the width of the safety wall.

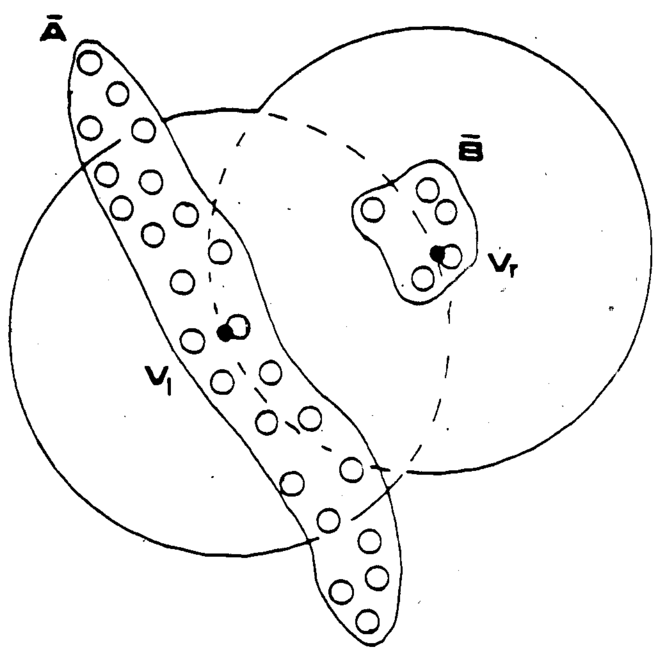


Figure 5–8 Situations Under Which the Three–Distance Clustering Method May be Deficient

The safety wall should theoretically follow the contours of both clusters in the n–dimensional measurement space (figure 5–9). The costs associated with the implementation of such a safety wall would soon prove to be insurmountable as the dimension of the measurement space increased. Feasible safety walls could employ one or more of the following proposals:

- dw may be a function of the cluster level
- dw may be a function of the number of objects in the cluster
- dw may be given by the largest distance between two points in the cluster.

Much consideration should be given towards the structure of the safety wall since it figures significantly in the final cluster configuration.

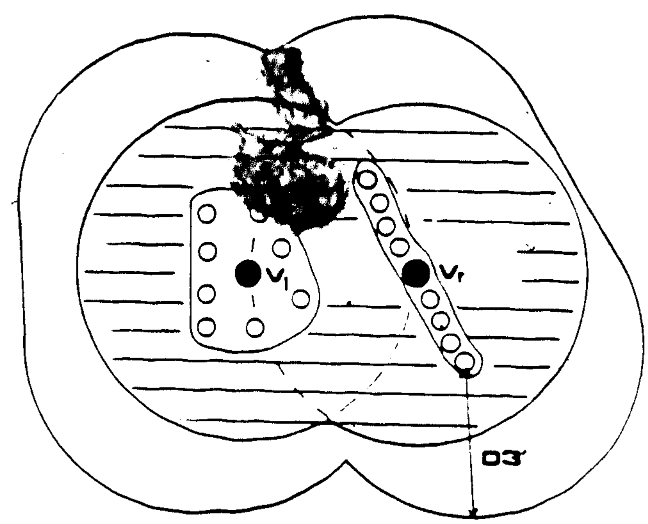


Figure 5-9 Illustration of an Ideal Safety Wall

VI. Program CLUSTREE for Three Distance Clustering

A self-contained routine entitled *CLUSTREE* has been written to implement the three distance clustering method on the Michigan Terminal System (MTS) operating system at the University of Alberta. The program differs in several respects from Zupan's three distance clustering routine entitled *THREE*. The most prominent difference is that CLUSTREE is written in a Pascal programming environment; Zupan's routine is written in DEC's Fortran IV language. There are several reasons for implementing the routine in Pascal instead of Fortran, the main one being that Pascal allows dynamic representation of data structures in addition to the usual contiguous representation. Dynamic variables differ from static variables in that they are created (and may be destroyed) during program execution; static variables are created when the program is initially compiled. The problem with contiguous representations of data structures, which employ static variables, is that the maximum size of the data structures must be known in advance such that they can be declared in the routine. For small data sets, much of the space that has been allocated for the data structures is not used, and hence is wasted space; for large data sets, overflow conditions can result when insufficient amounts of space have been allocated for the data structures. Dynamic representation of the data structures result in a correct amount of memory allocation during run time as dictated by the routine. Thus, memory is not wasted and overflow conditions can not occur.

Another advantage to writing the routine in Pascal is that the algorithm ideally lent itself to the structured Pascal environment. The employment of additional variable types that Pascal allows greatly improved the legibility as well as the efficiency of the routine. In addition, CLUSTREE was written such that no additional programming would be required on the part of the user; program THREE was written as a subroutine for which the user must supply a main program.

Under certain circumstances, the user may be interested only in identifying a limited number of clusters. For this purpose, a *dump* feature has been incorporated into the routine. This feature requires the user to specify the maximum height of the tree, which in effect constrains the maximum number of clusters identified by the routine. Since the clustering costs are related to the length of the tree, implementation of the dump option can result in a considerable cost savings.

CLUSTREE can cluster as many objects as the system memory permits. On MTS, this is well in excess of 5000 objects (the CLUSTAN package can only cluster a maximum of 1000 objects). The maximum dimension that the measurement space can span is 50. Again, it should be noted that as the dimension of the measurement space increases, the cost associated with clustering the objects increases coincidingly.

A. Discussion of the CLUSTREE Routine

The CLUSTREE routine consists essentially of a group of interlinked Pascal procedures. This type of modular design facilitates any changes or additions that may be required.

1. Clustree Procedures

The CLUSTREE routine is based around a framework of five central procedures: *DECISS, DIST, FUNC, SEARCH,* and *DELETE.* The DECISS procedure directs the traversal of the objects through the tree. The three distances that DECISS requires at each vertex are calculated by the DIST procedure. As new objects are added to the vertices, procedure FUNC updates the vertex representation according to the algorithm previously discussed. Once all of the objects have been allocated, procedure SEARCH tests the retrieval capability of the tree. The objects that cannot be successfully retrieved are then removed from the existing tree structure by the DELETE procedure. The above sequence is controlled by the main program and is repeated until either 100% retrieval capability is attained or the maximum number of iterations is reached. This process is summarized in the flowchart illustrated in figure 6-1.

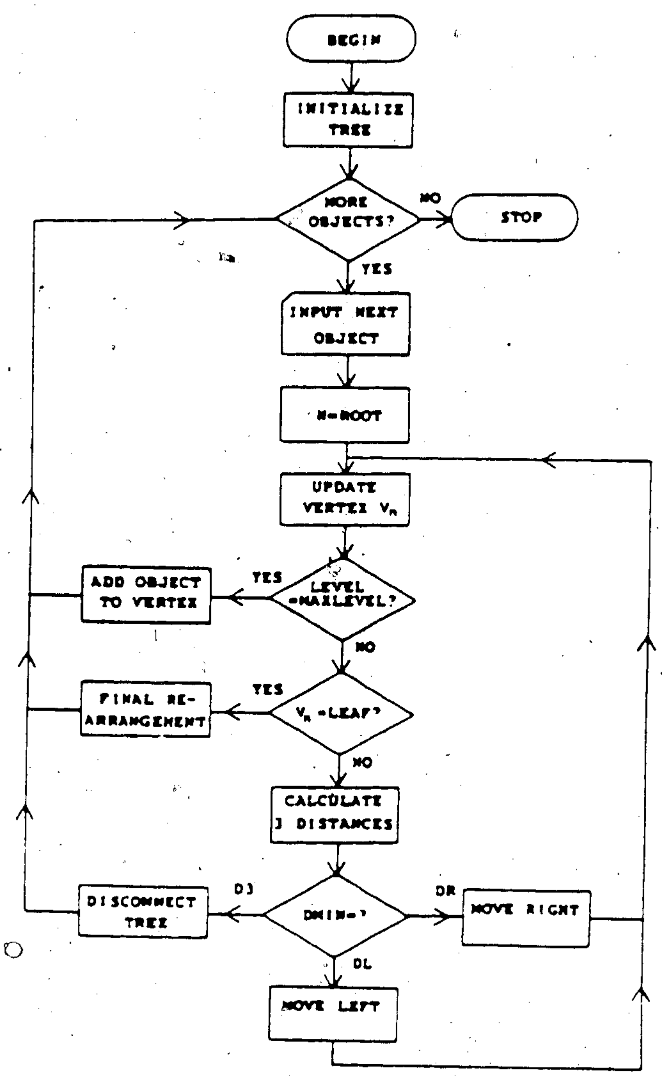


Figure 6–1 Flowchart of the Clustering Algorithm

The remaining procedures perform secondary functions such as output formatting. Many of these procedures are implemented through the use of the recursion feature available in the Pascal programming environment.

2. Input - Output

The routine communicates with the user through the use of four text files (two input files, two output files). Both an input and an output file are automatically assigned by the routine to the output device that the user is currently using; the remaining input and output files are assigned to *scards* and *sprint*, respectively.

a. CLUSTREE Input Requirements

The CLUSTREE routine requires two sources of input:

i) data located on a MTS file

ii) interactive data for which the user is prompted

The file data must be organized in the following manner:

Line 1 - *no. of objects (n) and dimension of measurement space (m)*

Line 2 - *Obj(1,1) Obj(1,2) ... Obj(1,m)*

Line 3 - *Obj(2,1) Obj(2,2) ... Obj(2,m)*

. . . .

. . . .

Line n+1 - *Obj(n,1) Obj(n,2) ... Obj(n,m)*

In addition to the file data, the user is queried by the routine for: the maximum level to which the tree can grow, the maximum number of iterations for the update procedure, the type of distance metric to be implemented, and the type of output that is required. The distance metric is specified in terms of an integer ranging from 1 to 5 which corresponds to one of the distance measures listed in table 6-1.

Table 6-1 Distance Metrics Facilitated by CLUSTREE

Number	Name	Formulation
1	Manhattan	$d(X_i, X_j) = \sum_m \lvert X_{im} - X_{jm} \rvert$
2	Euclidean	$d(X_i, X_j) = [\sum_m (X_{im} - X_{jm})^2]^{1/2}$
3	Minkowski	$d(X_i, X_j) = [\sum_m (X_{im} - X_{jm})^m]^{1/m}$
4	Chebyshev	$d(X_i, X_j) = \underset{m}{\text{maximum}} \lvert X_{im} - X_{jm} \rvert$
5	Cosine	$d(X_i, X_j) = [\sum_m (X_{im} \cdot X_{jm})] / [\sum_m (X_{im})^2 \cdot \sum_m (X_{jm})^2]$

b. CLUSTREE Output Specifications

The output produced by the Clustree routine consists of four stages. The first stage summarizes the results attained after each update iteration. The second stage describes the final tree configuration; it consists of a listing of the level, type, vector representation, and number of objects for each vertex in the tree. The first two stages are always outputted when the CLUSTREE routine is invoked, whereas the next two stages are optional. Stage three gives a listing of the actual elements present in various levels of the tree. The levels for which this type of output is to be produced is provided by the user via the interactive input mode. The final output stage, stage four, produces a two dimensional scatter plot of the clusters produced at a requested level of the tree. Again, the specific parameters required for the plot are entered interactively in response to prompts from the routine.

3. Run Commands

To run CLUSTREE on MTS, the user has a choice between two Pascal compilers: PascalVS and PascalJB. In tests, the PascalJB compiler proved to be much more efficient then the PacsalVS compiler, and in addition, provided superior error diagnostics. All of the CLUSTREE runs were thus implemented using the PascalJB compiler. However, since the compiler is not currently available on the system library, the user must issue the following set of commands to run the routine:

R JMB:PASCALJB SCARDS=CLUSTREE SPUNCH=-LOAD

R -LOAD+JMB:PJBLIB SCARDS=INPUT SPRINT=OUTPUT

where:

- (-LOAD) is a temporary file that stores the compiled version of CLUSTREE
- INPUT contains the file data required by the CLUSTREE routine
- OUTPUT is the file into which all of CLUSTREE results are directed.

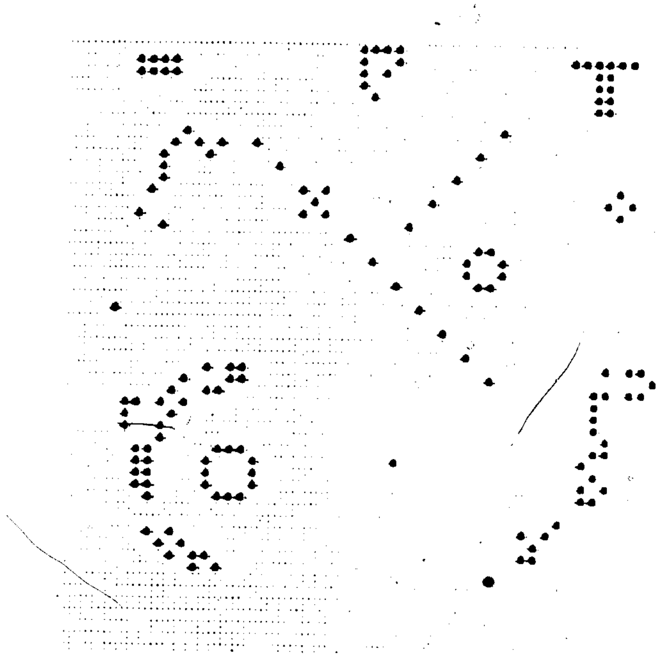
To run CLUSTREE using the PascalVS compiler, the JMB:PASCALJB and JMB:PJBLIB terms in the above commands must be replaced with PASCALVS and PASCALVSLIB, respectively.

B. Examples Using The Three Distance Clustering Procedure

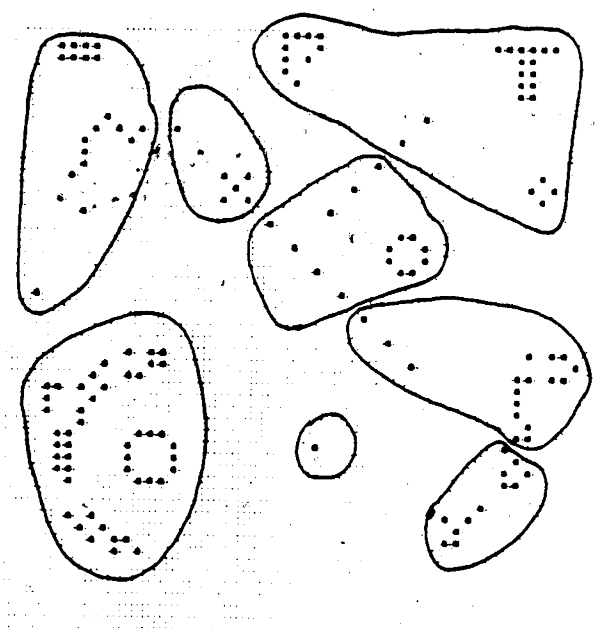
The three distance clustering technique is tested using two data sets. The first set contains Zupan's sample data and is chosen strictly to demonstrate that the routine runs properly. The second set contains Fisher's [1936] iris data. Since many clustering routines are tested using the iris data, the credibility of the new clustering technique can be ascertained.

1. Sample Run 1

Zupan's sample data consists of 150 points located in two dimensional measurement space (figure 6–2a). Zupan chose the data set with the consideration of having a sufficient but not excessive number of points to properly test the routine. The CLUSTREE routine required three iterations to successfully allocate all of the objects. The results attained using CLUSTREE are identical to those produced by Zupan's routine. Clusters formed at the third level of the hierarchical tree are illustrated in figure 6–2b.



a)



b)

FIGURE 6-2 a) Zupan's Sample Data b) Clusters Identified by the CLUSTREE Routine

2. Sample Run 2

The second data set consists of a series of measurements involving three different flower species: *Iris Setosa*, *Iris Versicolor*, and *Iris Virginica*. The flowers are represented in four-dimensional measurement space on the basis of: sepal length, sepal width, petal length, and petal width. A random sample of 50 points from each Iris is used to test the validity of the new clustering technique as compared to techniques from the *CLUSTAN* and *SPSSx* packages. The data points are given in table III.

Table 6–2 Fisher's Iris Data

Iris Setosa				Iris Versicolor				Iris Virginica			
Sepal Length	Sepal Length	Petal Length	Petal Length	Sepal Length	Sepal Length	Petal Length	Petal Length	Sepal Length	Sepal Length	Petal Length	Petal Length
20.0	4.0	5.1	3.5	1.4	0.2	7.0	3.2	4.7	1.4	6.3	3.3
4.9	3.0	1.4	0.2	6.4	3.2	4.5	1.5	5.8	2.7	6.0	2.5
4.7	3.2	1.3	0.2	6.9	3.1	4.9	1.5	7.1	3.0	5.9	2.1
4.6	3.1	1.5	0.2	5.5	2.3	4.0	1.3	6.3	2.9	5.6	1.8
5.0	3.6	1.4	0.2	6.5	2.8	4.6	1.5	6.5	3.0	5.8	2.2
5.4	3.9	1.7	0.4	5.7	2.8	4.5	1.3	7.6	3.0	6.6	2.1
4.6	3.4	1.4	0.3	6.3	3.3	4.7	1.6	4.9	2.5	4.5	1.7
5.0	3	1.5	0.2	4.9	2.4	3.3	1.0	7.3	2.9	6.3	1.8
4.4	2.9	1.4	0.2	6.6	2.9	4.6	1.3	6.7	2.5	5.8	1.8
4.9	3.1	1.5	0.1	5.2	2.7	3.9	1.4	7.2	3.6	6.1	2.5
5.4	3.7	1.5	0.2	5.0	2.0	3.5	1.0	6.5	3.2	5.1	2.0
4.8	3.4	1.6	0.2	5.9	3.0	4.2	1.5	6.4	2.7	5.3	1.9
4.8	3.0	1.4	0.1	6.0	2.2	4.0	1.0	6.8	3.0	5.5	2.1
4.3	3.0	1.1	0.1	6.1	2.9	4.7	1.4	5.7	2.5	5.0	2.0
5.8	4.0	1.2	0.2	5.6	2.9	3.6	1.3	5.8	2.8	5.1	2.4
5.7	4.4	1.5	0.4	6.7	3.1	4.4	1.4	6.4	3.2	5.3	2.3
5.4	3.9	1.3	0.4	5.6	3.0	4.5	1.5	6.5	3.0	5.5	1.8
5.1	3.5	1.4	0.3	5.8	2.7	4.1	1.0	7.7	3.8	6.7	2.2
5.7	3.8	1.7	0.3	6.2	2.2	4.5	1.5	7.7	2.6	6.9	2.3
5.1	3.8	1.5	0.3	5.6	2.5	3.9	1.1	6.0	2.2	5.0	1.5
5.4	3.4	1.7	0.2	5.9	3.2	4.8	1.8	6.9	3.2	5.7	2.3
5.1	3.7	1.5	0.4	6.1	2.8	4.0	1.3	5.6	2.8	4.9	2.0
4.6	3.6	1.0	0.2	6.3	2.5	4.9	1.5	7.7	2.8	6.7	2.0
5.1	3.3	1.7	0.5	6.1	2.8	4.7	1.2	6.3	2.7	4.9	1.8
4.8	3.4	1.9	0.2	6.4	2.9	4.3	1.3	6.7	3.3	5.7	2.1
5.0	3.0	1.6	0.2	6.6	3.0	4.4	1.4	7.2	3.2	6.0	1.8
5.0	3.4	1.6	0.4	6.8	2.8	4.8	1.4	6.2	2.8	4.8	1.8
5.2	3.5	1.5	0.2	6.7	3.0	5.0	1.7	6.1	3.0	4.9	1.8
5.2	3.4	1.4	0.2	6.0	2.9	4.5	1.5	6.4	2.8	5.6	2.1
4.7	3.2	1.6	0.2	5.7	2.6	3.5	1.0	7.2	3.0	5.8	1.6
4.8	3.1	1.6	0.2	5.5	2.4	3.8	1.1	7.4	2.8	6.1	1.9
5.4	3.4	1.5	0.4	5.5	2.4	3.7	1.0	7.9	3.8	6.4	2.0
5.2	4.1	1.5	0.1	5.8	2.7	3.9	1.2	6.4	2.8	5.6	2.2
5.5	4.2	1.4	0.2	6.0	2.7	5.1	1.6	6.3	2.8	5.1	1.5
4.9	3.1	1.5	0.2	5.4	3.0	4.5	1.5	6.1	2.6	5.6	1.4
5.0	3.2	1.2	0.2	6.0	3.4	4.5	1.6	7.7	3.0	6.1	2.3
5.5	3.5	1.3	0.2	6.7	3.1	4.7	1.5	6.3	3.4	5.6	2.4
4.9	3.6	1.4	0.1	6.3	2.3	4.4	1.3	6.4	3.1	5.5	1.8
4.4	3.0	1.3	0.2	5.6	3.0	4.1	1.3	6.0	3.0	4.8	1.8
5.1	3.4	1.5	0.2	5.5	2.5	4.0	1.3	6.9	3.1	5.4	2.1
5.0	3.5	1.3	0.3	5.5	2.6	4.4	1.2	6.7	3.1	5.6	2.4
4.5	2.3	1.3	0.3	6.1	3.0	4.6	1.4	6.9	3.1	5.1	2.3
4.4	3.2	1.3	0.2	5.8	2.6	4.0	1.2	5.8	2.7	5.1	1.9
5.0	3.5	1.6	0.6	5.0	2.3	3.3	1.0	6.8	3.2	5.9	2.3
5.1	3.8	1.9	0.4	5.6	2.7	4.2	1.3	6.7	3.3	5.7	2.5
4.8	3.0	1.4	0.3	5.7	3.0	4.2	1.2	6.7	3.0	5.2	2.3
5.1	3.8	1.6	0.2	5.7	2.9	4.2	1.3	6.3	2.5	5.0	1.9
4.6	3.2	1.4	0.2	6.2	2.9	4.3	1.3	6.5	3.0	5.2	2.0
5.3	3.7	1.5	0.2	5.1	2.5	3.0	1.1	6.2	3.4	5.4	2.3
5.0	3.3	1.4	0.2	5.7	2.8	4.1	1.3	5.9	3.0	5.1	1.8

The following analyses were performed on the Iris data:

- a *quick-cluster* analysis from the SPSSx package
- an analysis using the CLUSTREE program with the 'dump' option in effect
- hierarchy, relocate, and density analyses from the CLUSTAN package
- hierarchy, relocate and density analyses in conjunction with principal component analysis from the CLUSTAN package.

A summary of all of the classifications produced by the various routines is given in table 6-3.

Table 6-3 Classification of Fisher's Iris Data

Clustering Procedure	**PCA**	**# of Correct Class.**	**Cost (CPU seconds)**
Hierarchy (Ward's)	Yes	135	1.2
Relocate	Yes	135	1.4
Density	Yes	144	1.4
Hierarchy (Ward's)	No	124	1.1
Relocate	No	128	1.3
Density	No	107	1.4
Quick Cluster	-	135	0.4
CLUSTREE	-	139	0.2

None of the clustering techniques was able to successfully cluster all of the Iris's. The most accurate classification was produced by the density procedure in conjunction with principal component analysis (pca); only six Iris's were misclassified. The results generated from the hierarchy and relocate procedures in conjunction with pca were not as favourable, since each resulted in 15 misclassifications. The costs associated with the generation of these results were significantly higher than those incurred using either the *CLUSTREE* or *Quick-Cluster* algorithms.

As table 6-3 indicates, CLUSTREE performed well against the other packages. The routine was able to successfully classify 139 of the 150 Iris's, second only to the density analysis. More importantly, the cost associated with the classification was by far the lowest of all the analyses performed. Thus, CLUSTREE was able to attain a high level of efficiency without relinquishing accuracy.

The 'quick-cluster' procedure from the SPSSx package was selected mainly to test the overall efficiency of the CLUSTREE routine. It was developed as an efficient counterpart to standard clustering methods and is used to cluster large numbers of objects into predetermined numbers of groups. Quick-cluster was able to correctly classify 135 Irises at a level of efficiency that was surpassed only by the CLUSTREE routine.

The most dismal results of all the trials were produced by the CLUSTAN package when pca was not specified. The hierarchy, relocate, and density procedures produced 124, 128, and 107 correct classifications, respectively. The costs associated with these results were almost as high as those incurred when principal component analysis was invoked. Hence, it can be deduced that unless one is thoroughly familiar with the CLUSTAN package, a great deal of time and effort can be spent in an attempt to generate satisfactory results. Also, if the purpose of the analysis is to classify objects whose inherent classification is not immediately known, then one may be at odds as to which CLUSTAN procedures to invoke. The CLUSTREE routine, on the other hand, requires the user to select only a distance metric; the routine can be directly implemented without any inherent knowledge of clustering on the part of the user. This can result in a substantial savings of time and money.

VII. Analysis of Ultrasonic Vocalizations

A. Methodology

1. Animals

Vocalizations were recorded from eleven rats (eight male, three female) of the Sprauge-Dawley strain (Biosciences, Edmonton, Alta.). For simplicity, the rats will be referred to by the numbering scheme listed in table 7-1.

TABLE 7-1 Rat Identification Chart

Number	Sex	Run Trial	Swim Trials
1	male	X	
2	male	X	
3	male	X	
4	male	X	
5	male		X
6	male	X	
7	male	X	X
8	male		X
9	female	X	
10	female	X	X
11	female		X

The rats were located in a environment in which controlled temperature (22($\pm$.5)°C) and light conditions (6:00 A.M.:6:00 P.M., light:dark) were maintained. They were housed in groups of two or three in standard size cages in which food and water were constantly available. All of the vocalizations were recorded during the 12 hour dark cycle.

2. Apparatus

The ultrasonic vocalizations emitted by the rats were recorded using a system which consists of two integral parts:

i) ultrasound detector

ii) digital ultrasound recorder

The ultrasound detector consists of a microphone sensitive to high frequencies and a variable range amplifier. It can operate in two different modes: tuned and broadband. In the tuned mode, the detector responds to sounds within a narrow frequency band selected by the user. The broadband mode permits the detection of signals located within a much larger frequency band. For all of the subsequent trials, the broadband mode was employed.

The digital ultrasound recorder, designed and tested by Paranjape[1983], is capable of recording vocalizations with frequencies up to 90 kHz. (accuracy for frequencies below 10 kHz. is not guaranteed). The recorder is connected directly to the output of the ultrasound detector. Once a call has been detected, the recorder samples the signal at pre-specified intervals and digitizes the amplitude and frequency information. The uncertainty for the amplitude and frequency measurements are 10% and 5%, respectively. The recorder then transmits the data to the university's main-frame computer using a modem and existing phone lines.

3. Data Collection Protocol

Rat vocalization parameters were recorded under a number of different stress situations. Many of these situations employed exercise as the major stimuli eliciting the stress response. Traditionally, invasive techniques (e.g., analyzing organ size, pulmonary function and blood serum cholesteral levels) have always been employed to measure the effects of exercise stress. The digital ultrasound recording system provides a ***real-time non-invasive*** alternative to these techniques. Recording exercise stress in terms of vocalizations virtually eliminates any possible interactions between the experimental design conditions and the data collection protocol.

a. Running Trials

One of the methods commonly employed to subject rats to exercise stress is running. However, running is usually employed as a means to investigate cardiac and metabolic functions. There is no record of anyone extensively analyzing vocalizations elicited in response to running. Vocalizations recorded during the running trials could be used to assess the levels of stress that the rats were experiencing. "(There is also the) possibility that the acoustic behavior of the rat could be an important dependant measure that can be applied to other research programs"[Nitchke, 1982].

The running conditions were modelled with the use of a rat exercise readmill. The treadmill rotates in a manner such that the rats must be in constant motion in order to avoid contact with a shock plate. The speed and slope can be varied according to the training regime.

In order to eliminate any possible interaction effects between the animals, all of the runs were conducted with only one rat running at any particular time. Thus, vocalizations recorded during the run could be attributed solely to the rat on the treadmill.

Three individual trials involving a total of eight rats were conducted over a period of eight months. One of the trials followed the two-week training schedule given in table 7-2; the remaining trials followed only the first week of the training schedule. Table 7-3 gives a comprehensive list of the animals involved with each trial along with the respective ages at which they were run. One additional trial was conducted in which rat #10 was ran for only one session at a speed of 30 m/min. for 120 min.

TABLE 7-2 Run Training Schedule

Day	Treadmill Speed (m/min)	Run Duration (min)
	WEEK 1	
1 (Mon)	20	10
2 (Tue)	20	15
3 (Wed)	25	15
4 (Thu)	25	20
5 (Fri)	30	20
	WEEK 2	
6 (Mon)	30	20
7 (Tue)	30	30
8 (Wed)	30	45
9 (Thu)	30	60
10 (Fri)	30	60

TABLE 7-3 Trial Run Parameters

Trial	Rats Involved	Age	Duration
1	#1, #2, #3, #4	10 weeks	1 week
2	#1, #3	18 weeks	2 weeks
3	#6, #7, #9	7 weeks	1 week

b. Swimming Trials

"Forced-swimming has frequently been employed as a convenient means of exposing small animals to the stress of exercise" [McArdle, 1967]. Through the course of his research, McArdle was able to quantify a negative correlation between body weight and the time to exhaustion.

It is common practice to load the rats with a certain percentage of body weight attached to their tails (similar to increasing the speed and/or slope of the treadmill during the running trials). However, McArdle noted that the loading effects could not be directly correlated with performance. Instead, he suggested that the addition of weight was indicant of the motivation of the animal. In addition to loading the animal, turbulent water conditions can be employed in lieu of the usual calm water conditions. Both techniques effectively reduce the time required to swim the rats to exhaustion.

A total of five swimming trials were conducted according to the conditions listed in table 7-4.

TABLE 7-4 Swimming Trials

Trial	Rat(s) Involved	% Load	Water Conditions
1	#5	0	calm
2	#5	3	calm
3	#7, #8	0	calm
4	#10, #11	0	calm
5	#11	0	turbulent

c. Other Trials

The rats were monitored for vocalizations under a number of different situations that did not directly involve exercise as the stressor.

i. Rats just completing their runs on the treadmill were transferred to rooms containing rats in a non-stressful environment. Selected rats in the room were then monitored for vocalizations.

ii. As previously stated, the rats were contained in groups of two or three to a cage. Thus, while one of rats was running on the treadmill, the remaining rat(s) were monitored for vocalizations.

iii. The simple gesture of turning on the treadmill may be a sufficient stimuli to provoke the rat vocalizations. Thus the rats were monitored for calls in response to noise generated by the treadmill.

iv. The rats were monitored for vocalizations in response to sounds artificially produced at previously recorded stress frequencies.

B. Data Analysis Techniques

1. Preliminary Steps

The digital ultrasound recorder formats the data as illustrated in figure 7-1. Each new call is identified by the sequence:

$$255\ 255\ 000\ 000\ t_1\ t_2$$

where 't_1 t_2' represents the interval during which the vocalizations were recorded.

Immediately following this sequence are a series of numbers which represent the amplitude and frequency values at various intervals during the call. This sequence is depicted below:

$$a_1\ f_1\ a_2\ f_2\ a_3\ f_3\ \ldots\ a_n\ f_n$$

The amplitude and frequency parameters of the call are each represented by an integer value that can vary between 0 and 255. The amplitude parameter measures the call amplitude in terms of mV.; the frequency parameter represents between 0 to 90 kHz. The number of amplitude and frequency values recorded for each call represents the duration of the call.

255	255	000	000	000	001	018	000	030	000	017	004	255	255	000
000	000	001	020	014	036	016	035	014	255	255	000	000	000	001
010	008	255	255	000	000	000	001	010	016	010	017	075	022	204
020	255	020	124	028	027	015	012	015	011	016	255	255	000	000
000	001	010	023	010	027	255	255	000	000	000	001	010	103	011
108	043	105	088	102	026	101	065	095	055	096	018	093	045	091
011	063	014	072	016	089	010	048	255	255	000	000	000	001	011
069	255	255	000	000	000	001	010	082	255	255	000	000	000	001
010	033	009	032	016	061	010	084	021	087	255	255	000	000	000
001	010	049	010	071	010	040	024	076	029	075	023	072	013	072
017	071	033	071	035	072	027	072	020	072	017	072	028	069	029
070	013	069	009	052	009	069	037	069	037	070	042	067	041	070
065	070	067	068	032	065	041	068	042	067	049	068	059	068	066
066	049	067	023	065	017	066	024	063	036	066	027	066	070	065
064	068	052	065	010	014	255	255	000	000	000	001	010	068	011
068	040	070	126	069	136	067	158	067	141	066	198	068	183	068
208	068	180	068	217	067	209	065	194	066	188	067	183	067	209
068	207	067	141	064	198	068	167	066	174	067	123	065	128	064
087	067	051	066	033	066	088	067	086	066	078	063	052	064	162
063	024	063	134	068	144	064	072	064	132	068	098	068	029	068
111	070	057	070	079	068	055	072	255	255	000	000	000	001	010
070	010	075	027	073	032	072	018	069	022	069	016	070	255	255
000	000	000	001	010	058	019	067	025	066	041	067	037	068	129
068	152	068	151	068	151	065	103	068	112	063	125	068	105	068
122	065	169	068	152	068	016	063	122	066	185	065	205	066	182
067	086	065	041	065	027	064	041	063	027	066	043	065	060	064
081	066	085	062	093	065	103	062	101	064	091	066	076	061	055
066	061	064	053	066	041	064	051	061	054	066	053	065	056	063
053	064	034	066	047	064	048	064	044	062	025	061	010	063	021

Figure 7-1 Digital Recorder Output

Before any analysis can take place, the datum sets must be formatted in order to make them compatible with existing analysis techniques. A Pascal routine entitled *READDATA* has been written for this purpose and is listed in Appendix 3. The new datum sets contain the following parameters:

- interval during which the call occurred
- call duration
- mean amplitude of the call
- mean frequency of the call
- standard deviation of the call amplitudes
- standard deviation of the call frequencies

The formated version of the data in figure 7-1 is given in figure 7-2.

call No.	interval	duration	mean ampl	mean freq	sdev ampl.	sdev freq
1	1	3	21.6667	1.3333	7.2342	2.3094
2	1	3	30.3333	14.6667	8.9629	1.1547
3	1	1	10.0000	8.0000	0.0000	0.0000
4	1	9	80.8889	18.7778	93.5073	4.2655
5	1	2	10.0000	25.0000	0.0000	2.8284
6	1	13	31.6923	89.6923	25.3390	17.9601
7	1	1	11.0000	69.0000	0.0000	0.0000
8	1	1	10.0000	82.0000	0.0000	0.0000
9	1	5	13.2000	59.4000	5.1672	26.5387
10	1	40	32.6500	65.8750	18.2399	10.7087
11	1	43	123.2791	66.8140	63.0594	2.0384
12	1	7	19.2857	71.1429	8.3009	2.2678
13	1	56	72.6786	64.1250	51.5788	4.1955
14	1	10	13.6000	60.4000	3.2387	4.3256
15	1	8	51.0000	66.1250	38.4299	1.9594
16	1	3	11.0000	62.6667	1.7321	3.0551
17	1	4	10.2500	61.2500	1.2583	2.8723
18	1	29	26.0000	63.8621	10.3751	4.9909
19	1	4	10.5000	64.7500	1.7321	1.8930
20	1	5	28.2000	71.8000	20.0674	1.0954
21	1	26	56.5769	66.3846	41.9468	1.6511
22	1	5	10.6000	62.8000	1.3416	4.3243
23	1	6	11.5000	67.5000	1.9748	5.0892
24	1	16	34.9375	69.0000	22.8312	1.4606
25	1	6	22.3333	71.1667	9.2448	1.6021
26	1	3	10.0000	69.6667	0.0000	2.0817

Figure 7-2 Formatted Data Output

2. ANALYSIS

The datum sets were initially analyzed for any correlations between the principle variables. Hierarchical log-linear models were then fitted to check for higher-order interactions. Both procedures were implemented through the use of the *SPSSx* package (the program listings are given in Appendix 4). The results of the correlation and hierarchical log-linear procedures were then employed to determine which variables should be used for the cluster analysis procedure.

The frequency components of the recorded calls were extensively analyzed. Each recording session was broken down into its constituent time intervals and the frequencies that were recorded within each interval were noted. Thus, it could be determined if frequencies of particular interest occurred either within specific intervals or randomly throughout the recording sessions.

In addition, *maps* of the vocalization space were recorded by employing two–dimensional gray plots and three–dimensional surface plots of the amplitude and frequency components of the calls.

C. Results

From the data collected, it can be shown that exercise with laboratory rats does cause stress. This stress can be quantified in terms of the high frequency vocalizations (21 – 28 kHz.) produced by the rats. Almost all of the data sets that were analyzed contained vocalizations at the known stress frequencies.

1. Running Trials

The running trials represented the most acute form of stress that the animals were subjected to during the data collection period. The high stress levels were reflected by the intensity of the vocalizations produced by the rats.

Correlation analysis of the datum sets was able to detect only limited interactions between the mean and standard deviation terms for both the amplitude and frequency variables. Detailed examination of the data revealed that the frequency standard deviation values were very small at the stress frequencies. This is substantiated by Scheidt[1979], who observed that stress calls show relatively little frequency modulation. Also, the stress call durations were significantly longer than the durations of the other calls. Results from the hierarchical log–linear analysis ruled out the possibility of any higher order interactions.

Cluster analysis was then applied to the data sets. Two different sets of variables were used to span the measurement space for the analysis. One of the measurement spaces consisted of the amplitude and frequency components of the call while the other consisted of the amplitude, frequency and duration variables. In almost all of the cases, cluster analysis was able to detect two distinct vocalization groups. One of the groups, located in the lower frequency band, can be at least partially attributed to noise generated by the treadmill. This was verified by recording the sounds produced by the treadmill alone. The remaining vocalizations in the first group are audible *squeals* produced by the rats. The higher frequency vocalization group was usually contained within a narrow frequency bandwidth, which in effect eludes to the stressful nature of these calls.

From the vocalizations recorded, it was evident that some of the rats would run better at the higher treadmill speeds than at the lower speeds. There could be two possible reasons for this apparent discrepancy. Brooks and White [1978] report that "at lower speeds and grades rats appear to utilize their power inefficiently and their running is accompanied by extraneous activity". However, since the rats were not run at the lower speeds for significantly long durations, it is more likely that the increase in performance can be attributed to learning on the part of the rats.

Detailed analysis of the cluster data revealed that each of the rats consistently vocalized at specific stress frequencies that varied for each rat. The majority of the stress calls were contained within a frequency range of 21 - 26 kHz, which corresponds well to the known stress call frequencies for these rats.

One of the rats, #4, would run for only a short period of time and would then refuse to run. Bedford et al. [1979] reported that it is common to expect a significant percentage of rats (10%) to refuse to run. They then attempted to correlate the unwillingness to run with biological attributes. "However, after examination of these rats for resting $\dot{V}o_2$, resting heart rate, heart mass, ligamentous strength, and muscle cytochrome oxidase activity, there were no statistically significant differences between animals willing and unwilling to run". Vocalizations produced by the rats could provide a means of distinguishing between willing and unwilling runners. Rat #4 produced some of the most intense stress calls that were recorded throughout the sessions. These vocalizations were not rigidly constrained within the narrow stress call bandwidth previously isolated. The lower frequency limit of these calls was identical to that of the stress calls, however the upper limit not well defined in that scattered vocalizations were recorded at frequencies up to 40 kHz.

Figures 7–4 to 7–35 illustrate the vocalization space of the calls produced by:

- rats #1 and #3 during their two week training period
- rats #2 and #4 during their one week training period

The gray plots were generated by the Pascal routine *GRAY* ; the surface plots were generated by the Pascal and Fortan routines *SURFACE* and *DISSPL* , respectively (the Fortran routine was required to link the Pascal program with a Fortran graphics package entitled *DISSPLA*). The gray plots follow the format illustrated in figure 7–3.

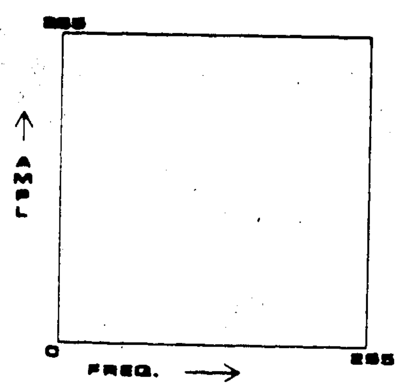


Figure 7–3 Gray plot format

Since the digital recorder encodes the amplitude and frequency information in terms of integers ranging from 0 to 255, the gray plots depict the recorder's measurement space.

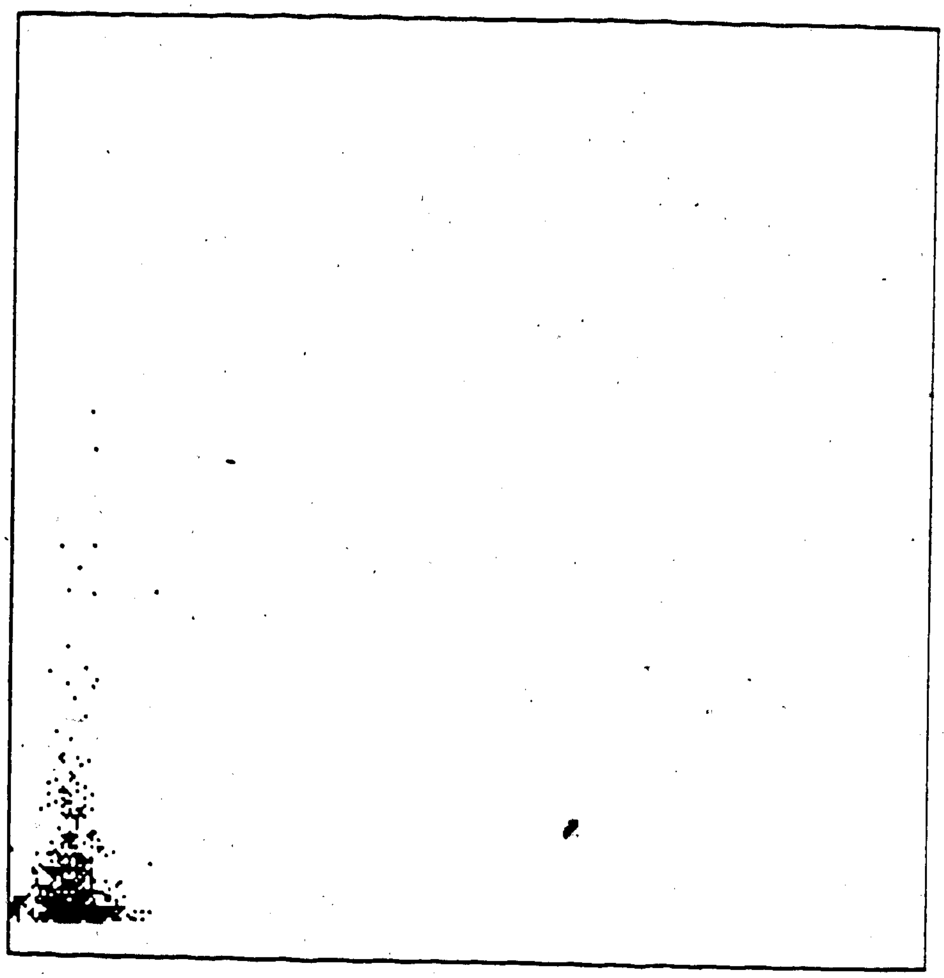


Figure 7-4 Vocalization Pattern Recorded for Rat #1 on Day 1.

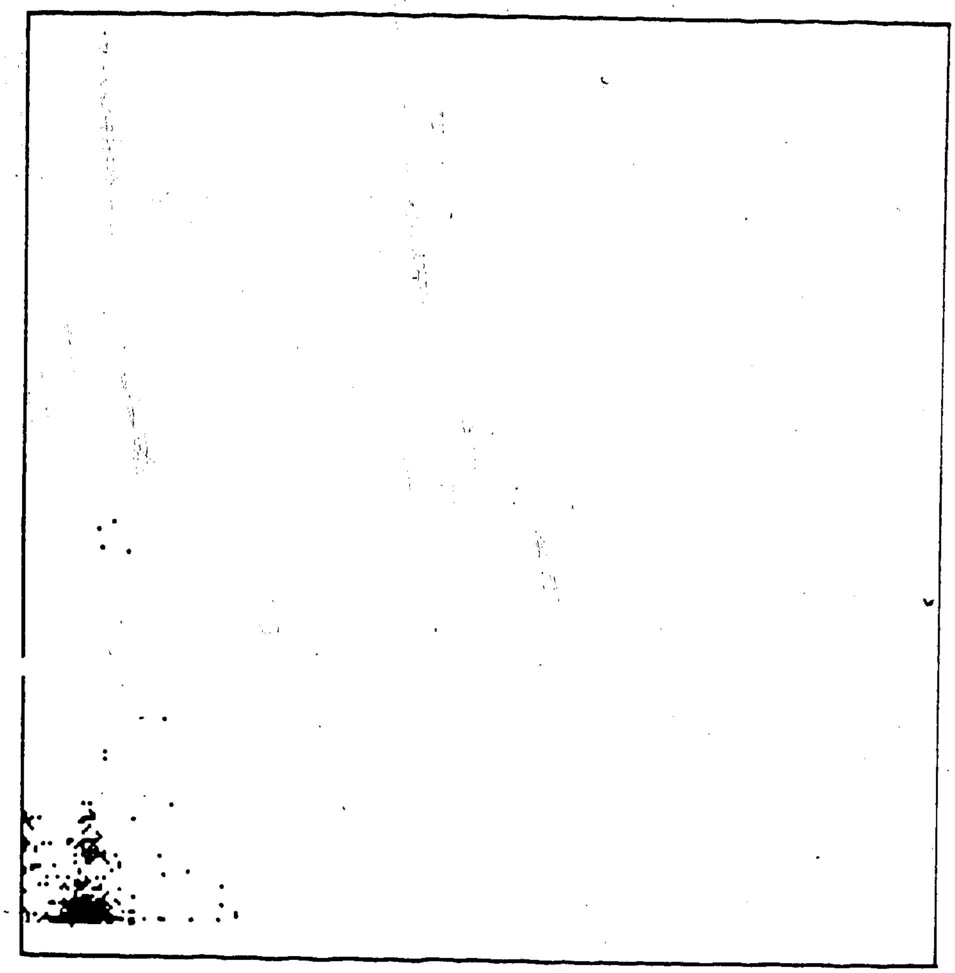


Figure 7-5 Vocalization Pattern Recorded for Rat #1 on Day 2.

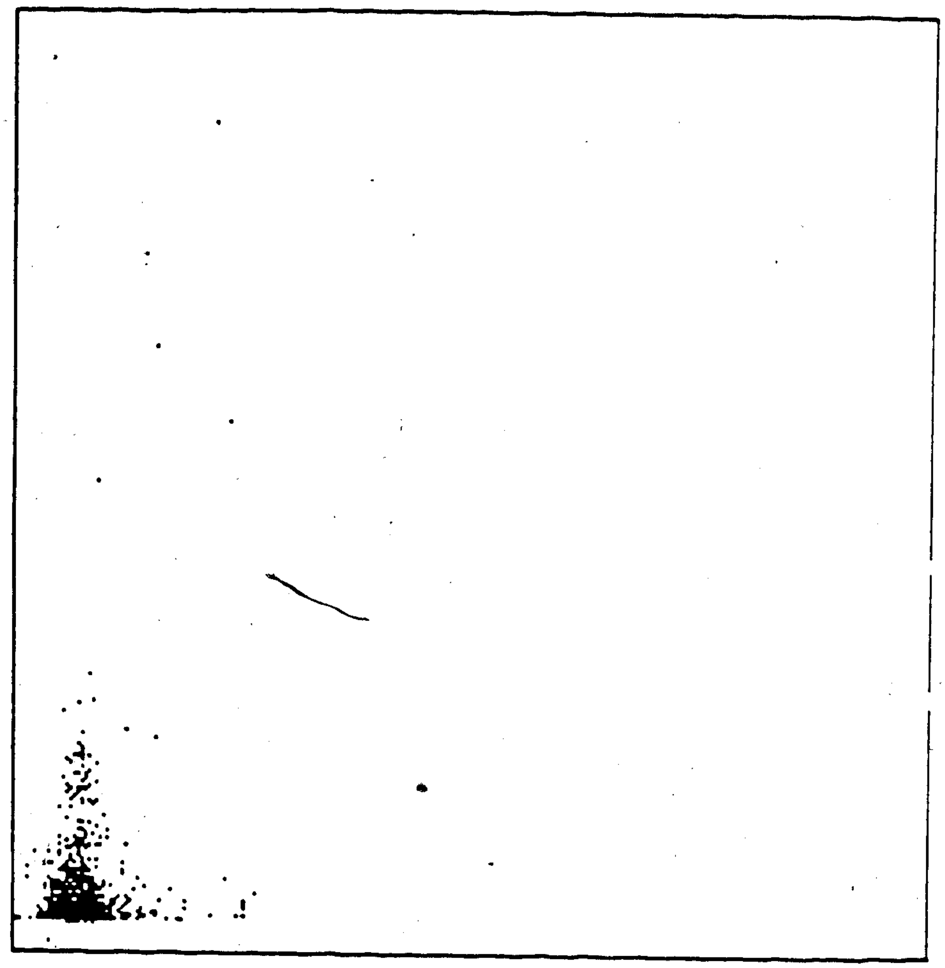


Figure 7-6 Vocalization Pattern Recorded for Rat #1 on Day 3.

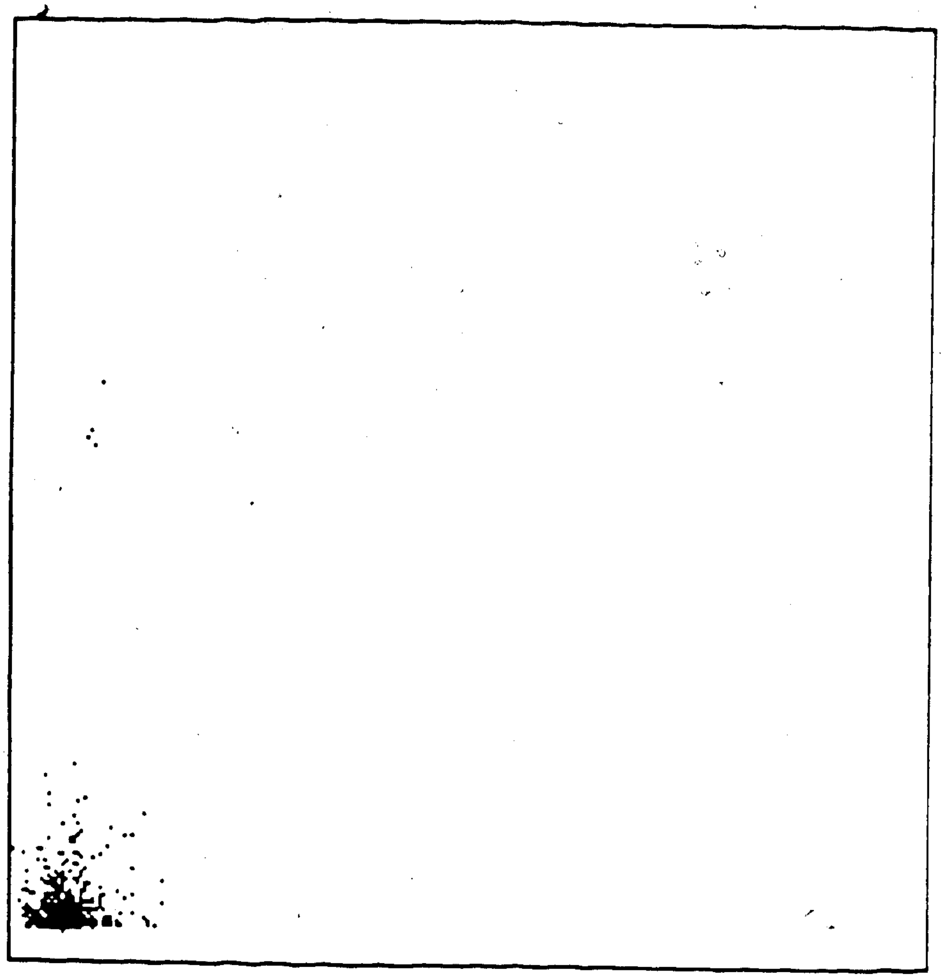


Figure 7-7 Vocalization Pattern Recorded for Rat #1 on Day 5.

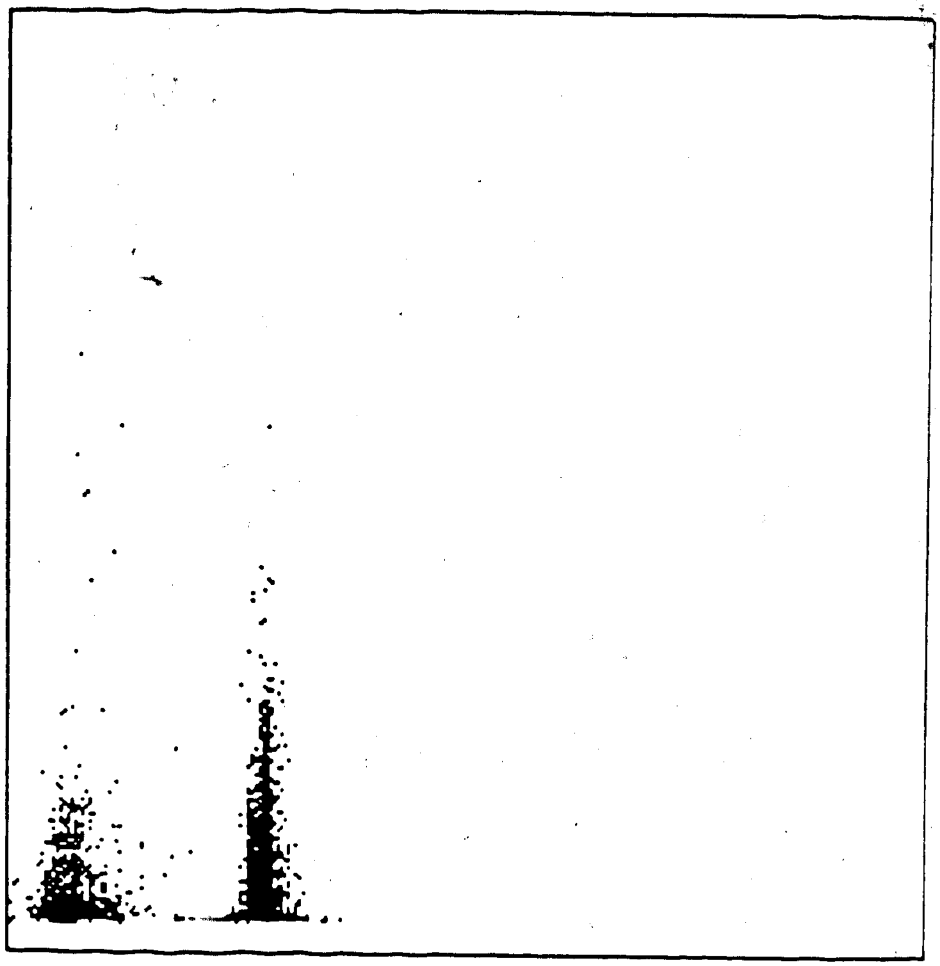


Figure 7–8 Vocalization Pattern Recorded for Rat #1 on Day 6[1].

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[1]Due to a power failure on the previous night, the light cycle was not in synchronous with the recording session. Thus, the stress calls can not be thought of as being exclusively elicited by the running situation.

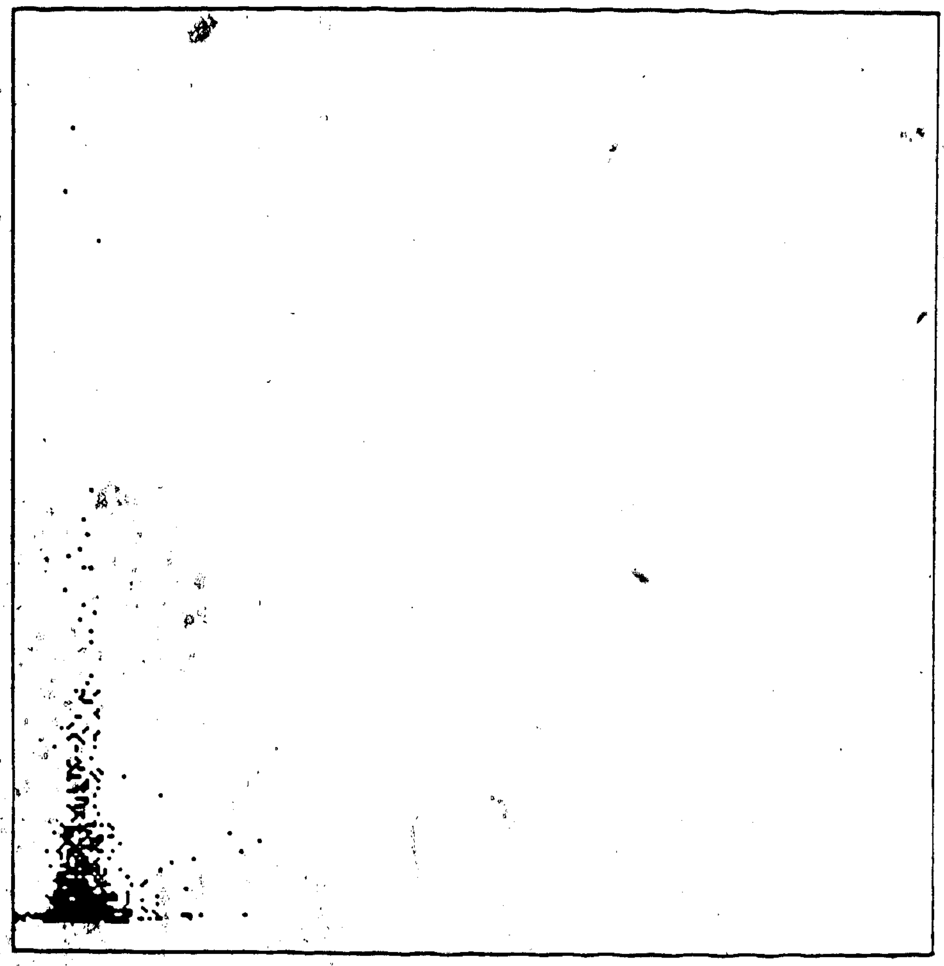

Figure 7-9 Vocalization Pattern Recorded for Rat #1 on Day 8.

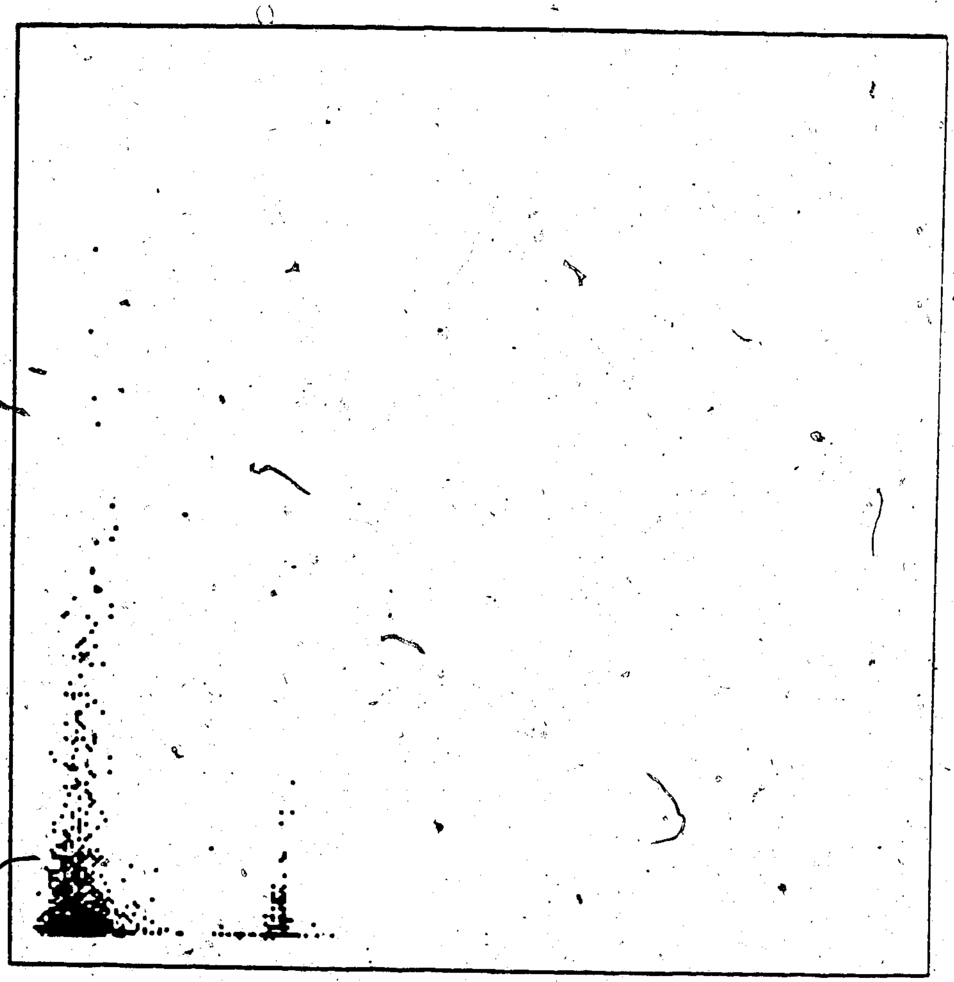

Figure 7-10 Vocalization Pattern Recorded for Rat #1 on Day 9.

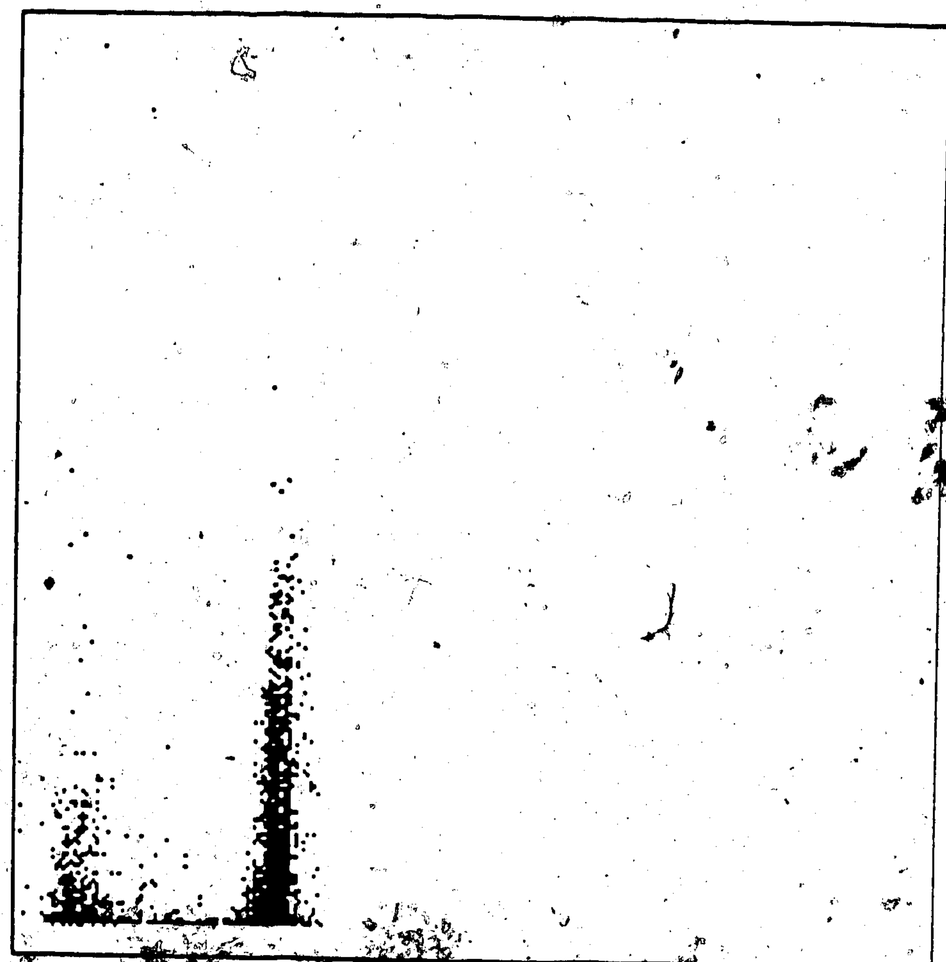

Figure 7-11 Vocalization Pattern Recorded for Rat #1 on Day 10.

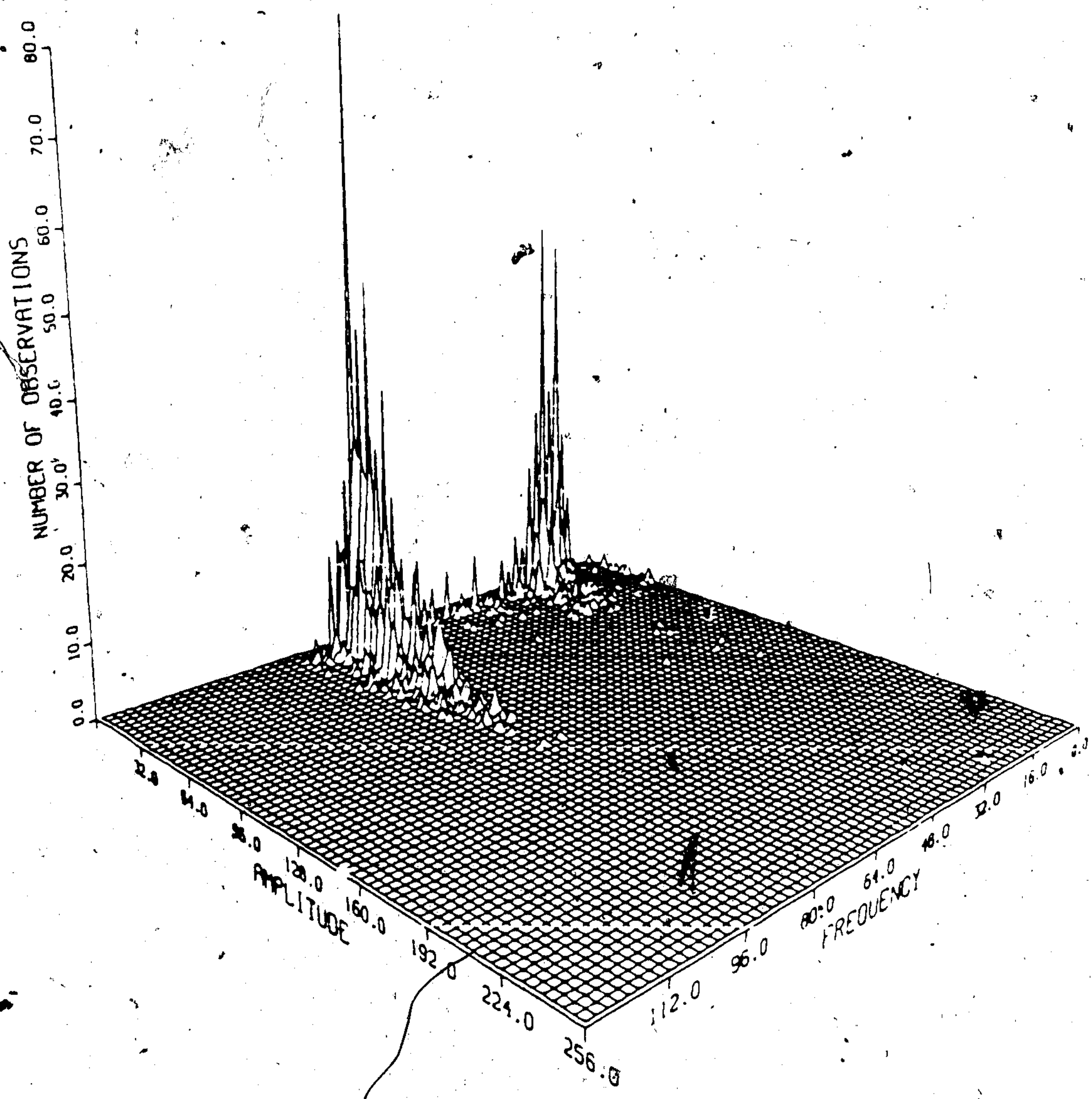

Figure 7-12 Surface plot of day 10 vocalizations for rat #1.

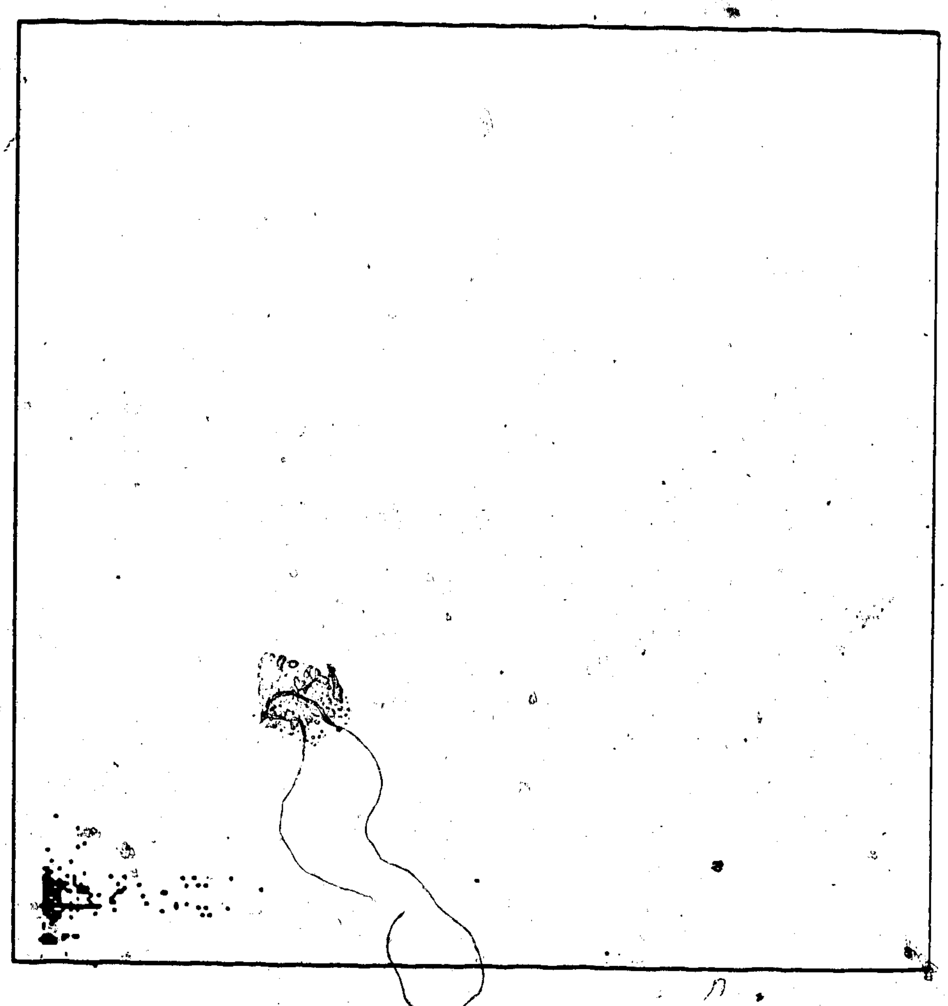

Figure 7-13 Vocalization Pattern Recorded for Rat # 3 on Day 1.

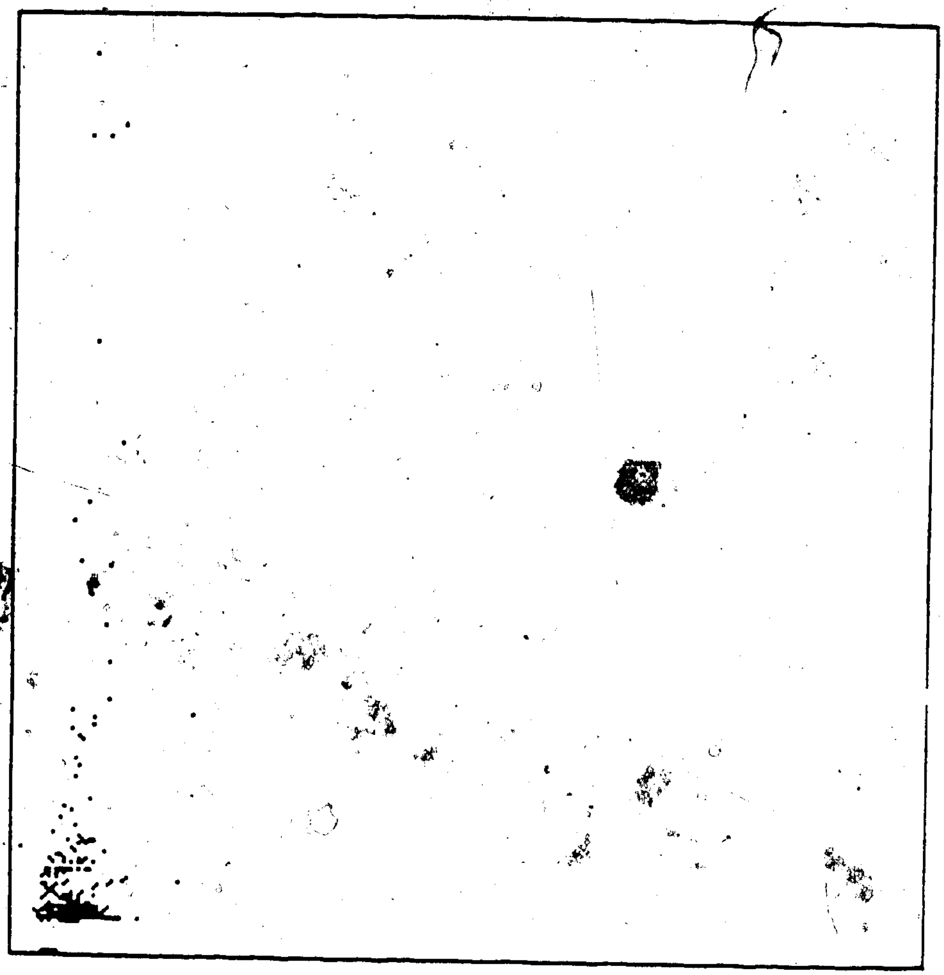

Figure 7-14 Vocalization Pattern Recorded for Rat # 3 on Day 2.

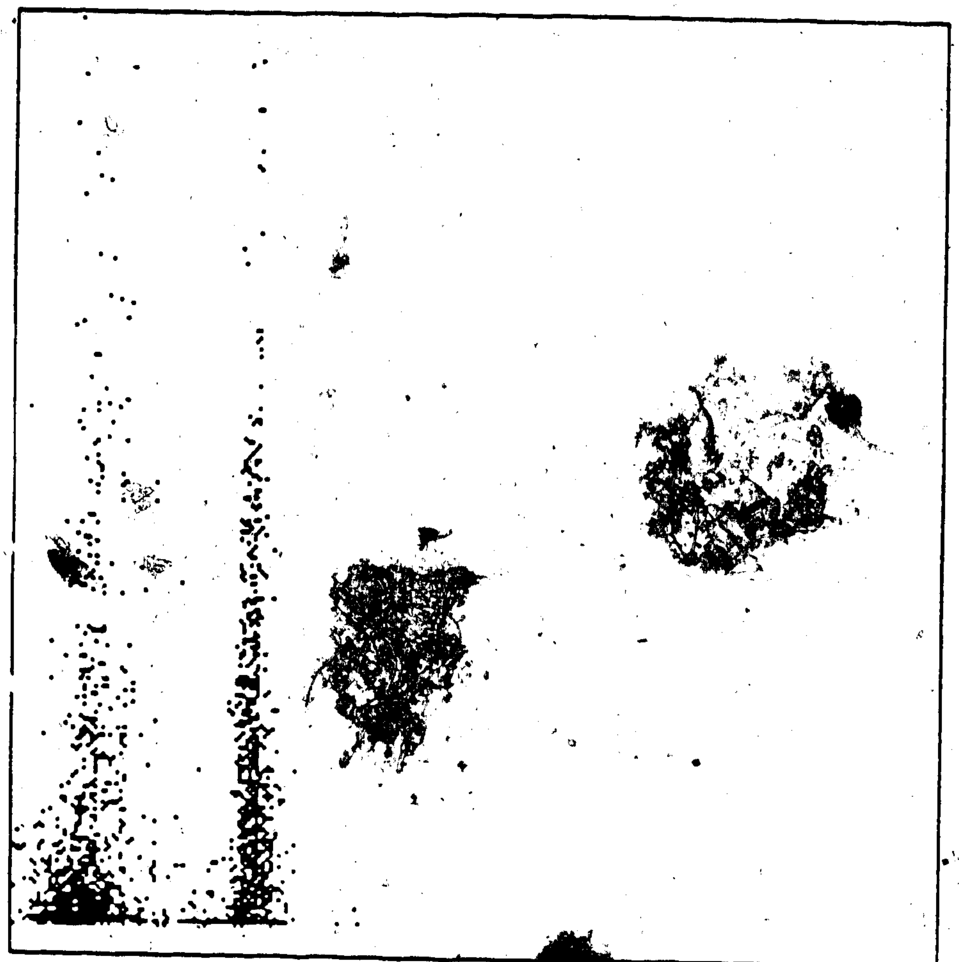

Figure 7-[illegible] Vocalization Pattern Recorded for Rat #3 on Day 3.

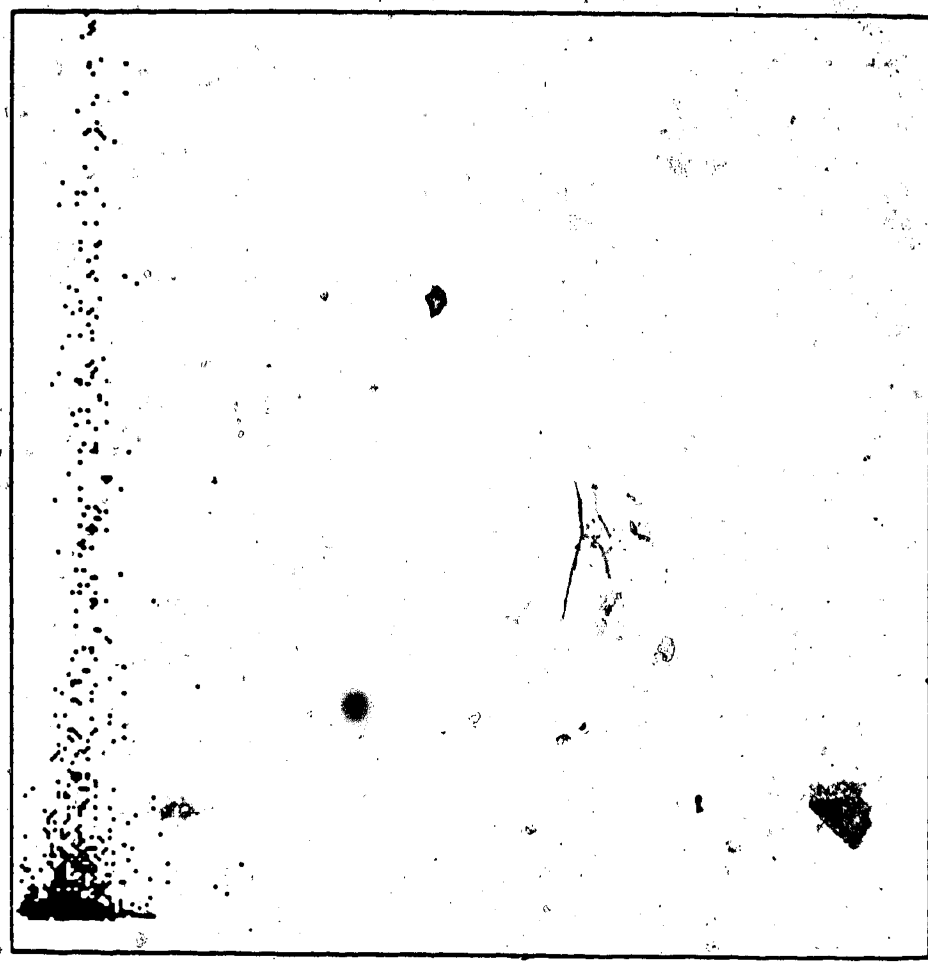

Figure 7-16 Vocalization Pattern Recorded for Rat #3 on Day 4.

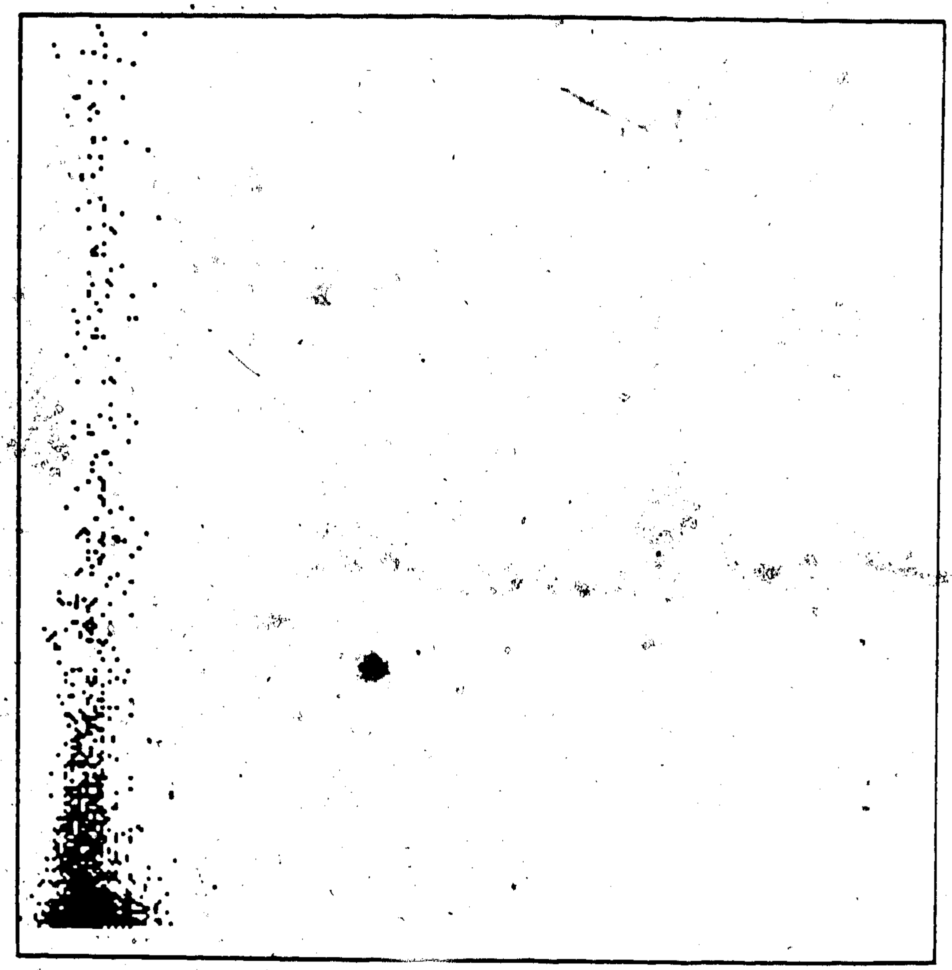

Figure 7-17 Vocalization Pattern Recorded for Rat #3 on Day 6.

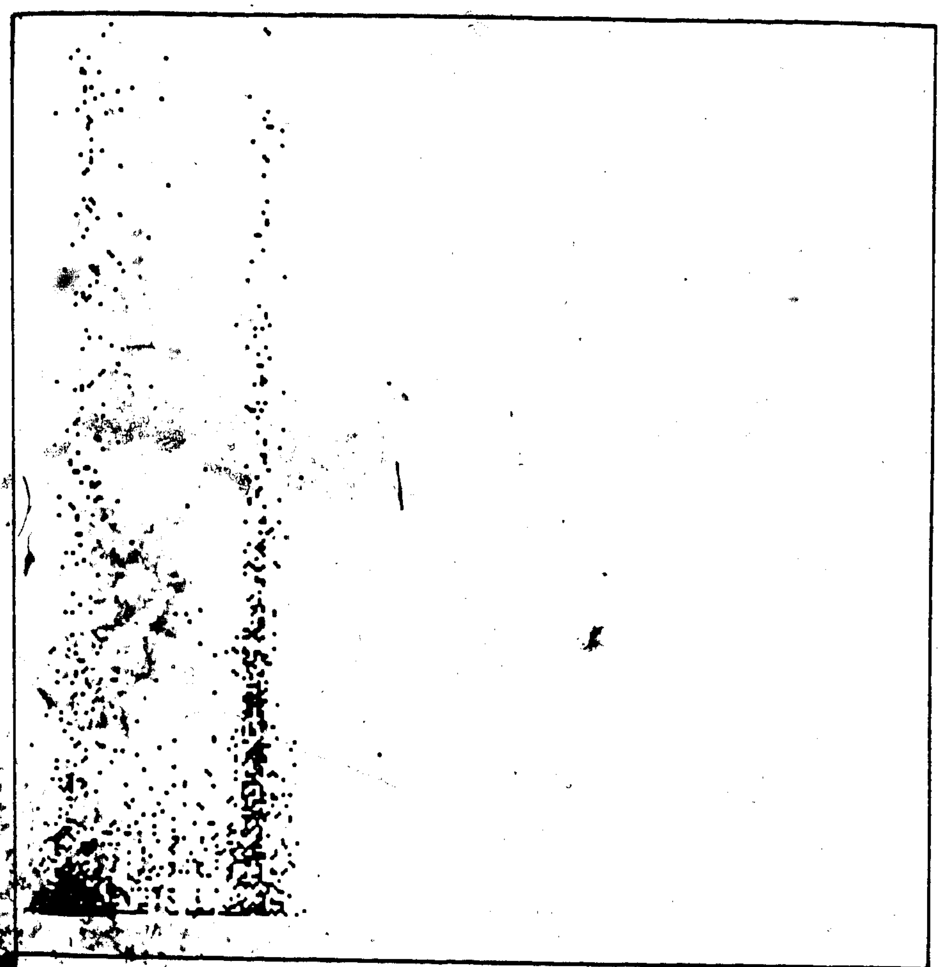

Figure 7-18 Vocalization Pattern Recorded for Rat #3 on Day 7.

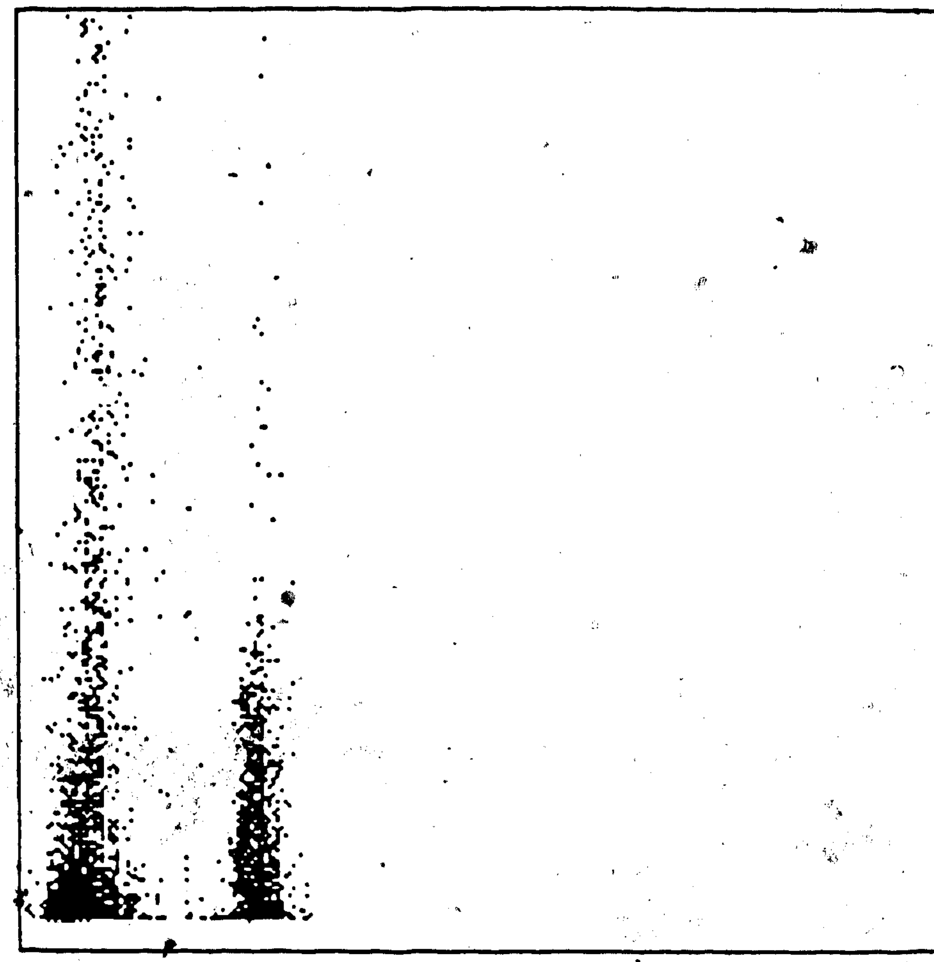

Figure 7-19 Vocalization Pattern Recorded for Rat #3 on Day 8.

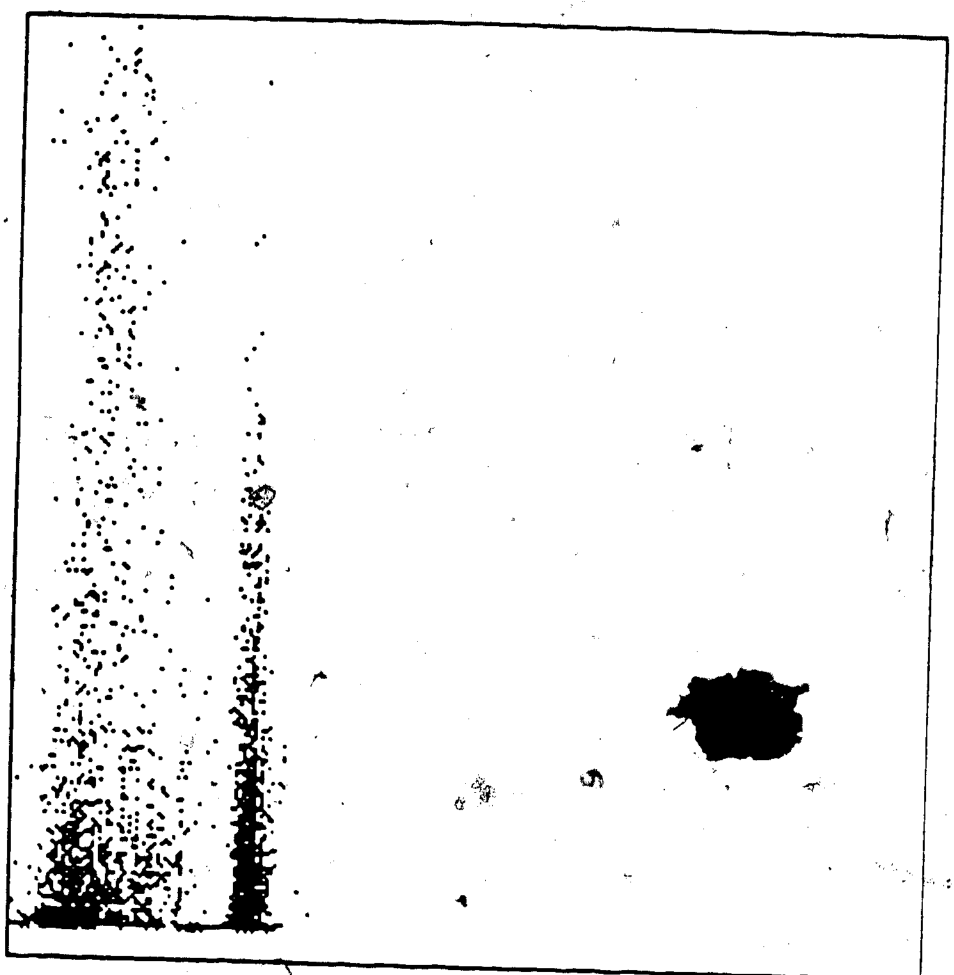

Figure 7-20 Vocalization Pattern Recorded for Rat #3 on Day 9.

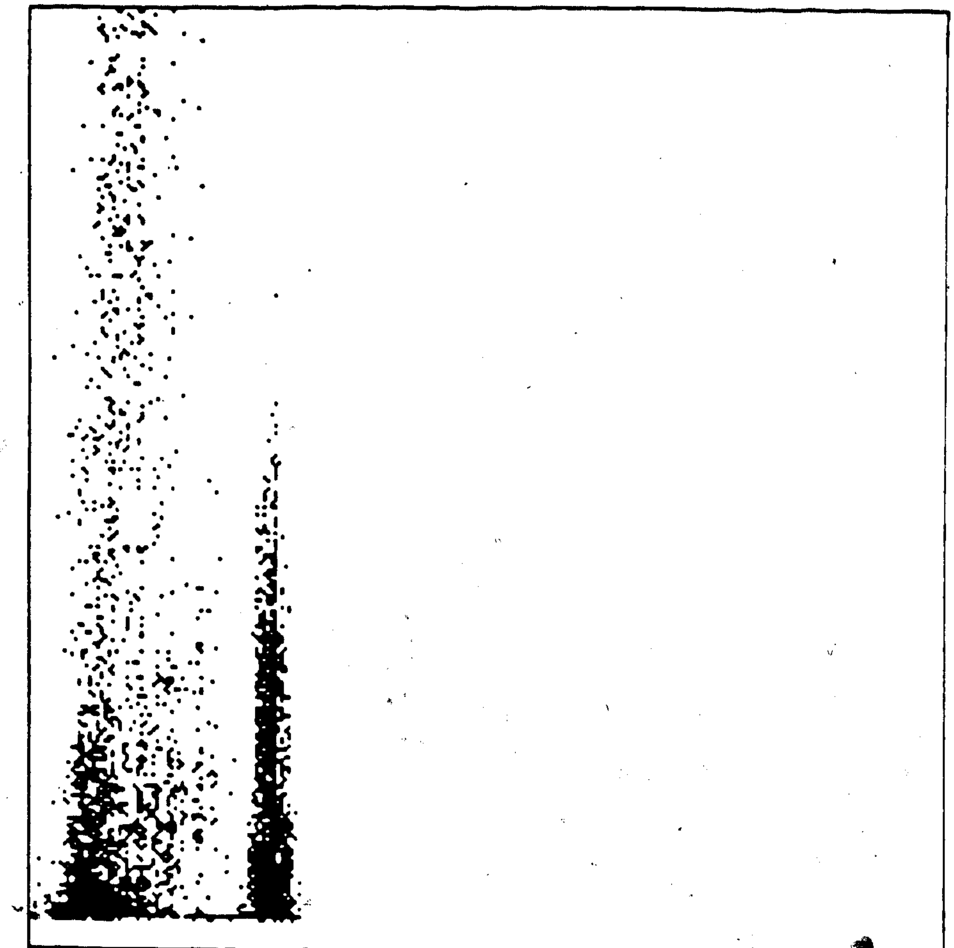

Figure 7-21 Vocalization Pattern Recorded for Rat #3 on Day 10.

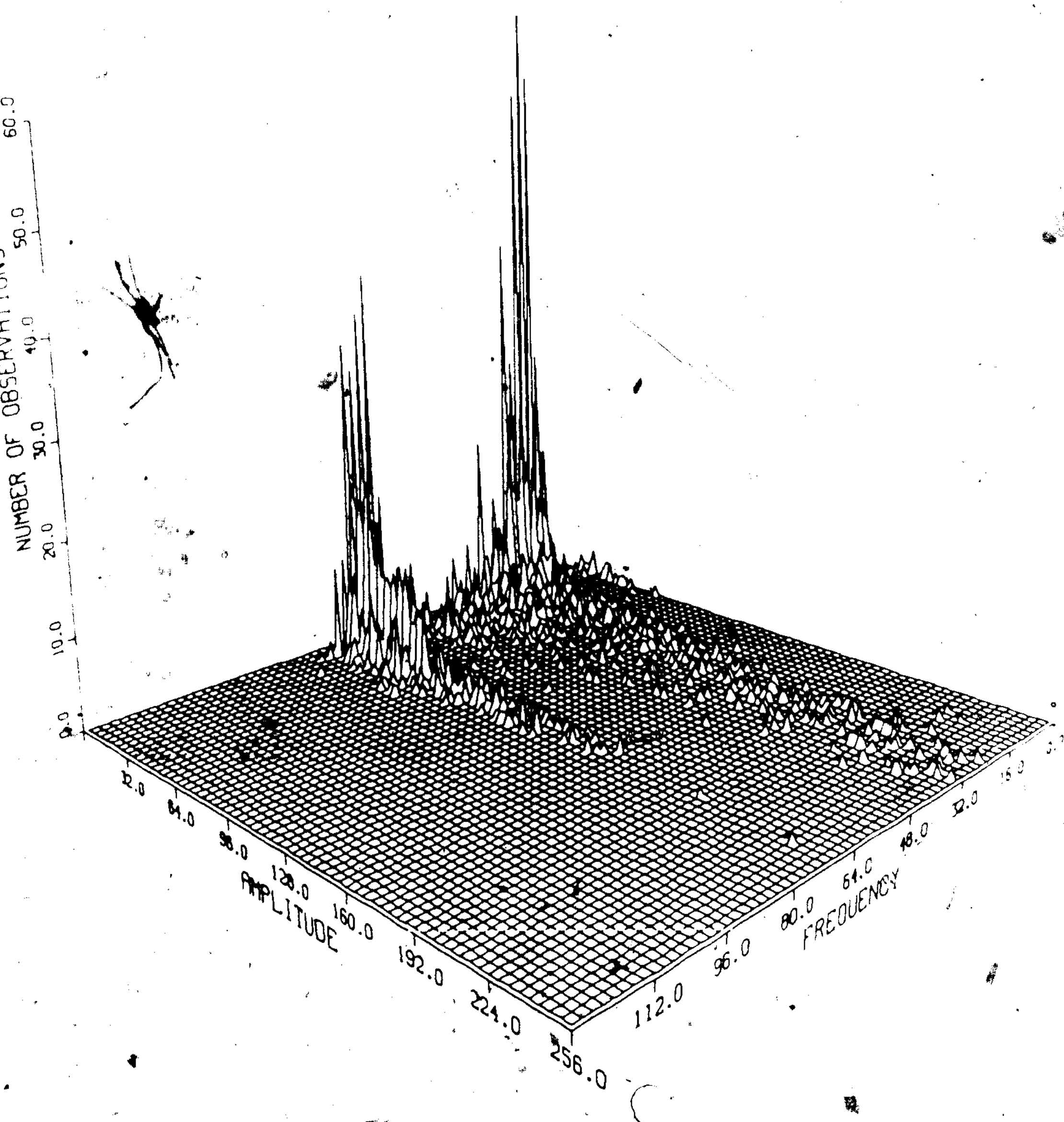

Figure 7–22: Surface plot of day 10 vocalizations for rat # 3.

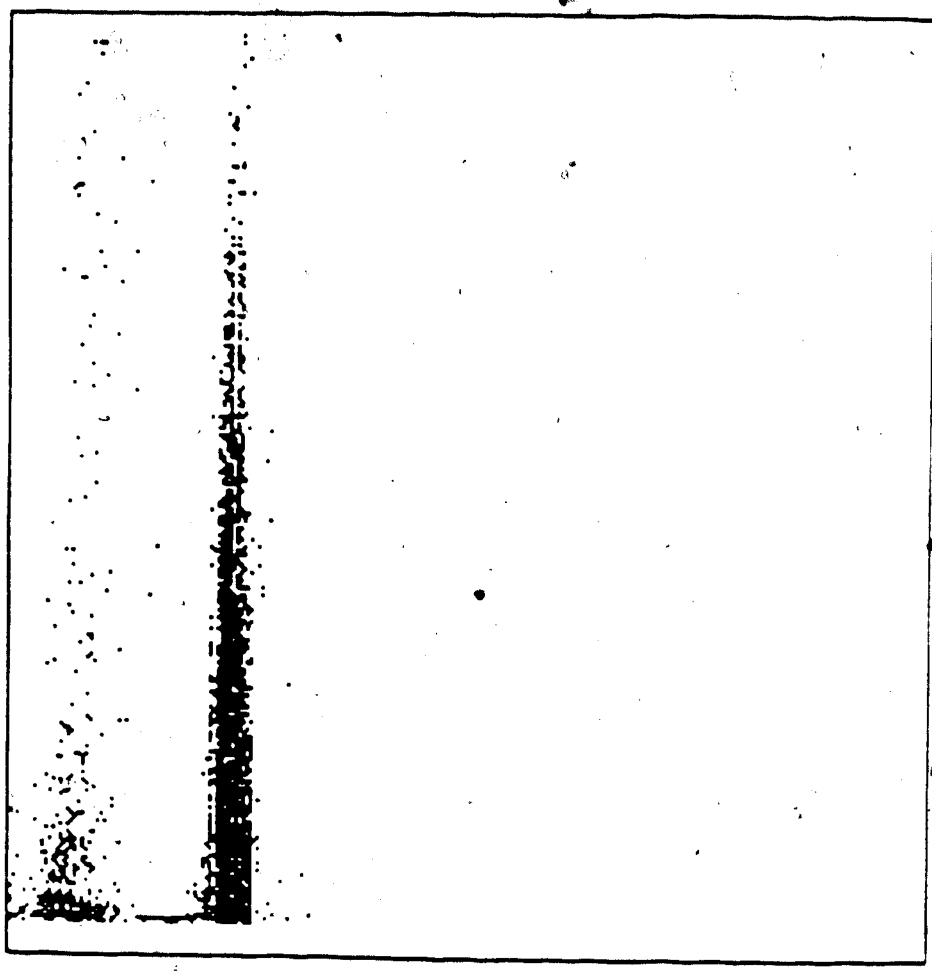

Figure 7-23 Vocalization Pattern Recorded for Rat #2 on Day 1.

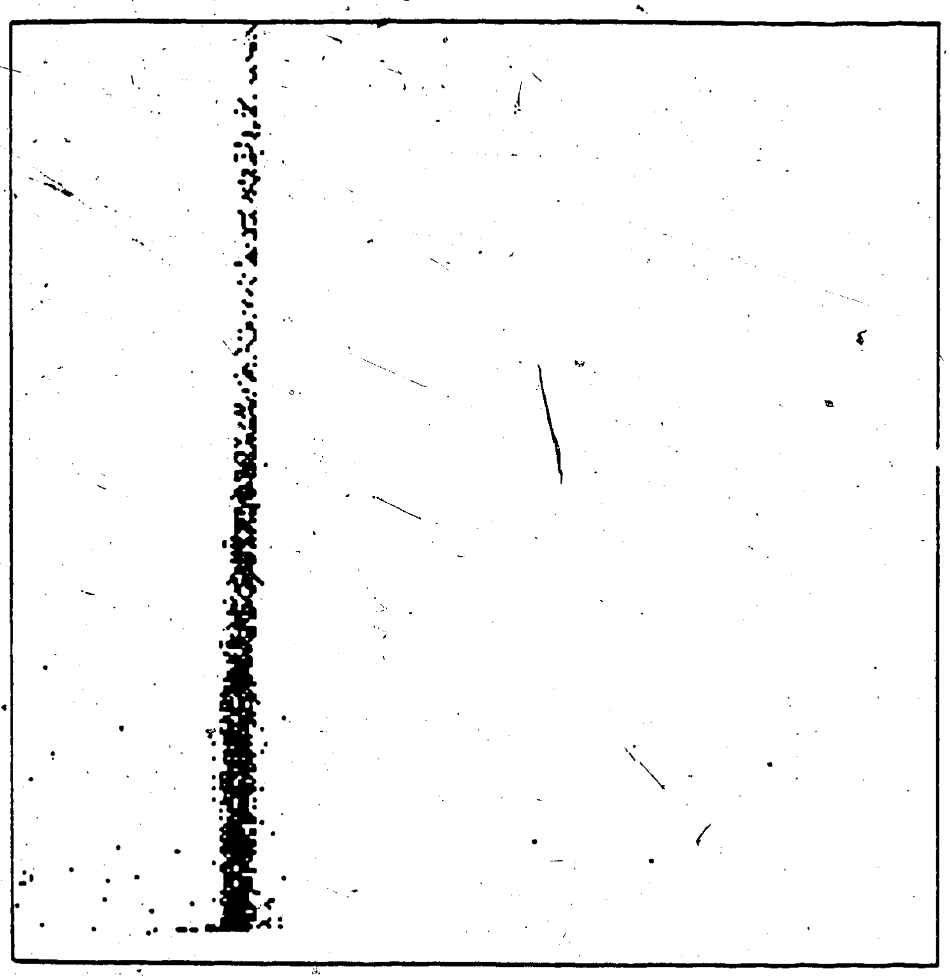

Figure 7-24 Vocalization Pattern Recorded for Rat #2 on Day 2.

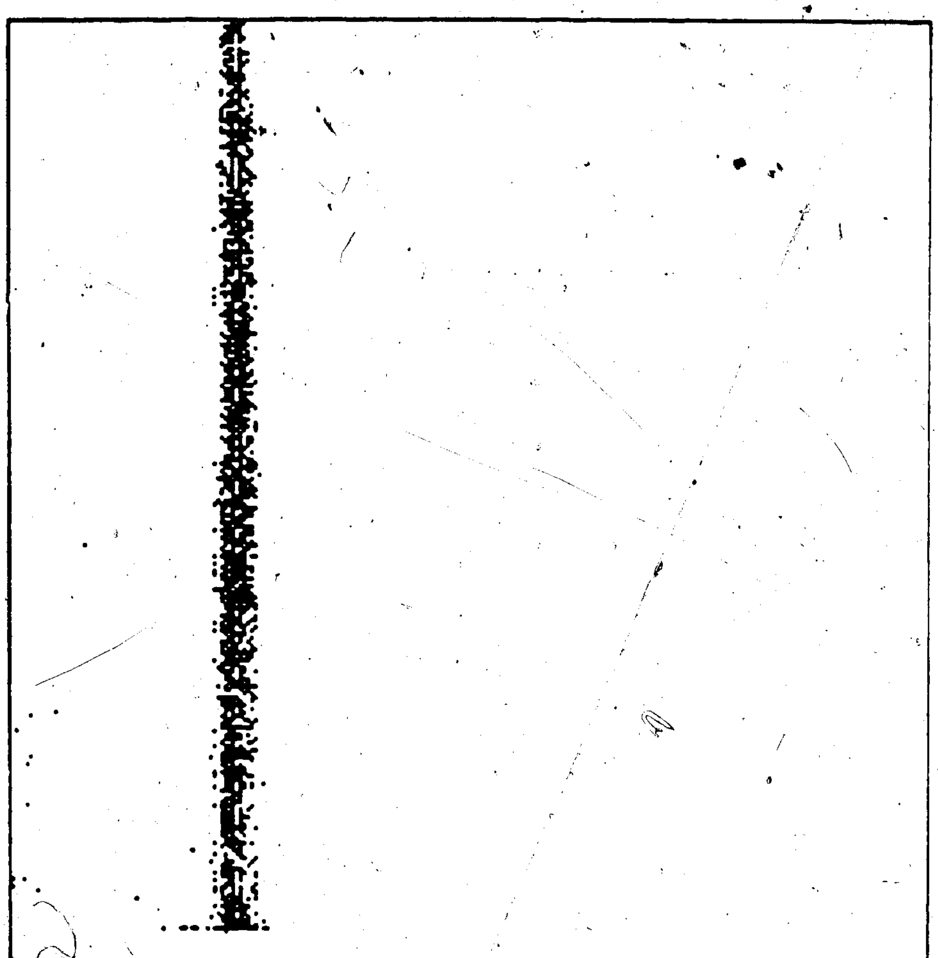

Figure 7-25 Vocalization Pattern Recorded for Rat #2 on Day 3.

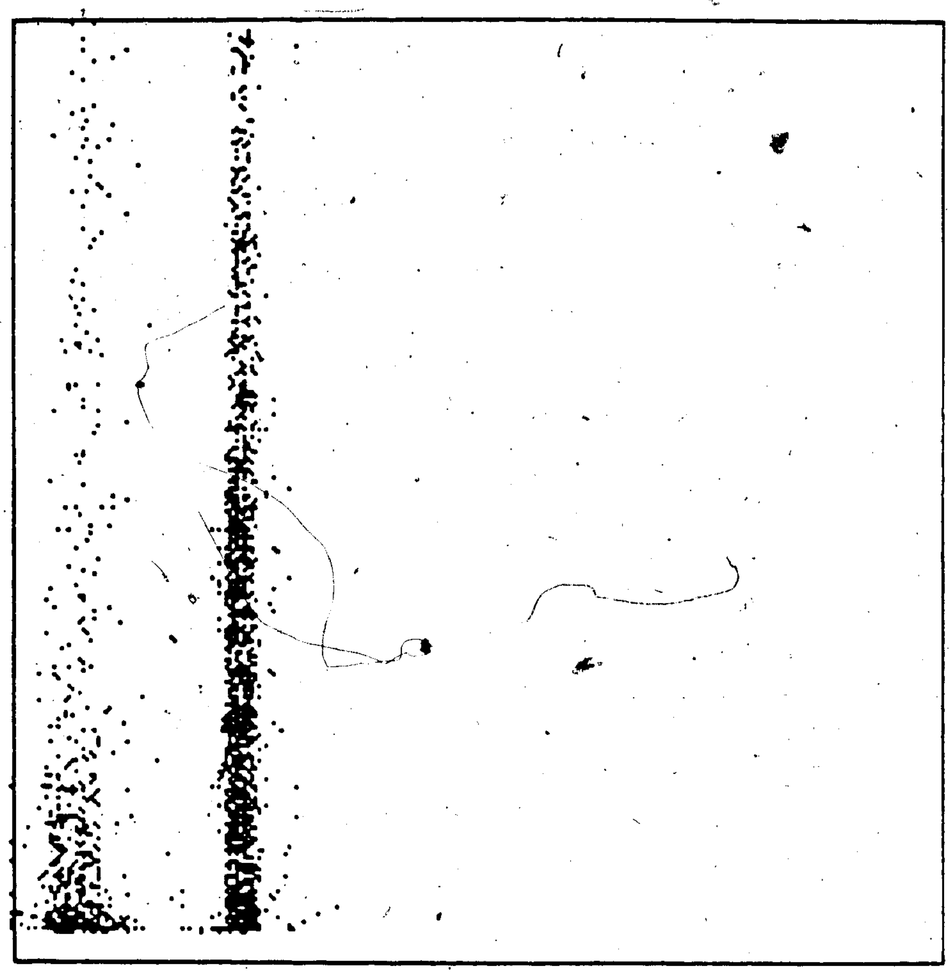

Figure 7-26 Vocalization Pattern Recorded for Rat #2 on Day 4.

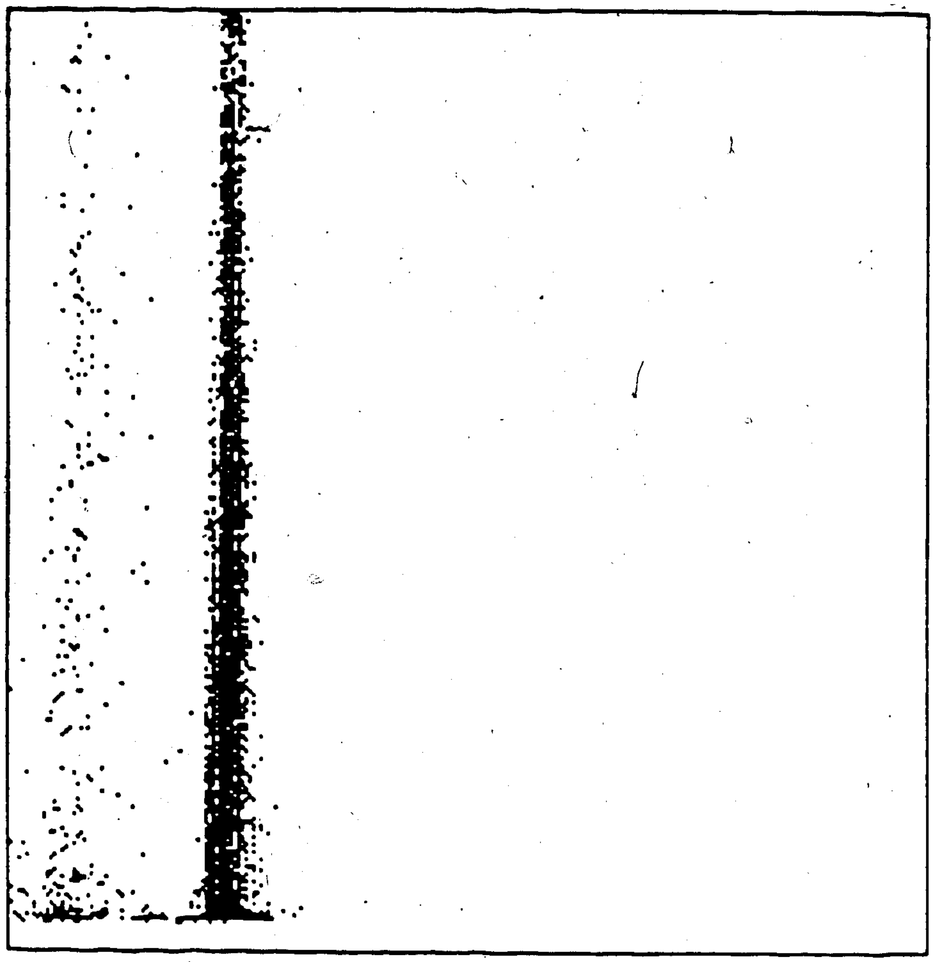

Figure 7-27 Vocalization Pattern Recorded for Rat #2 on Day 5.

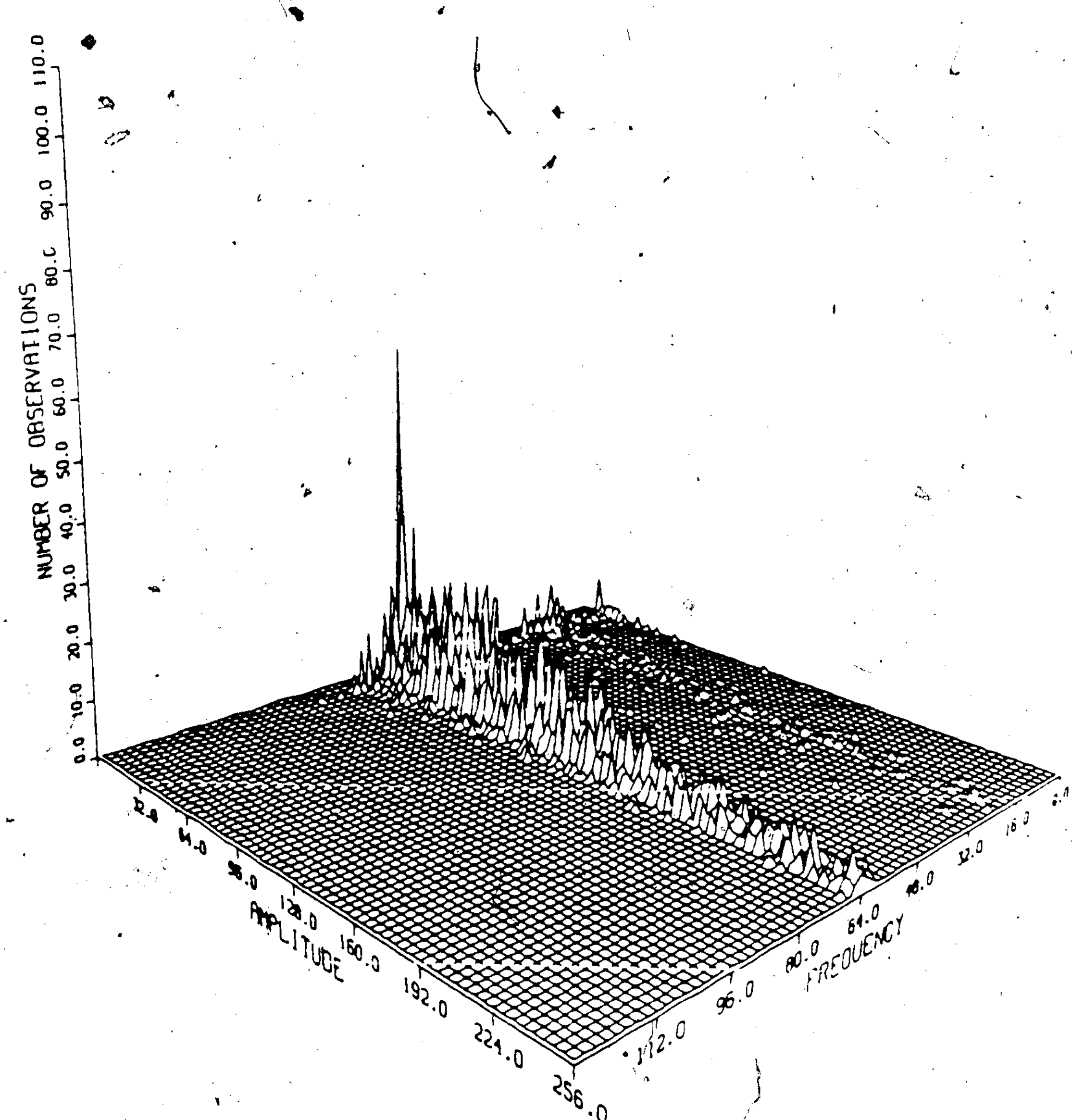

Figure 7-28 Surface plot of day 5 vocalizations for rat #2.

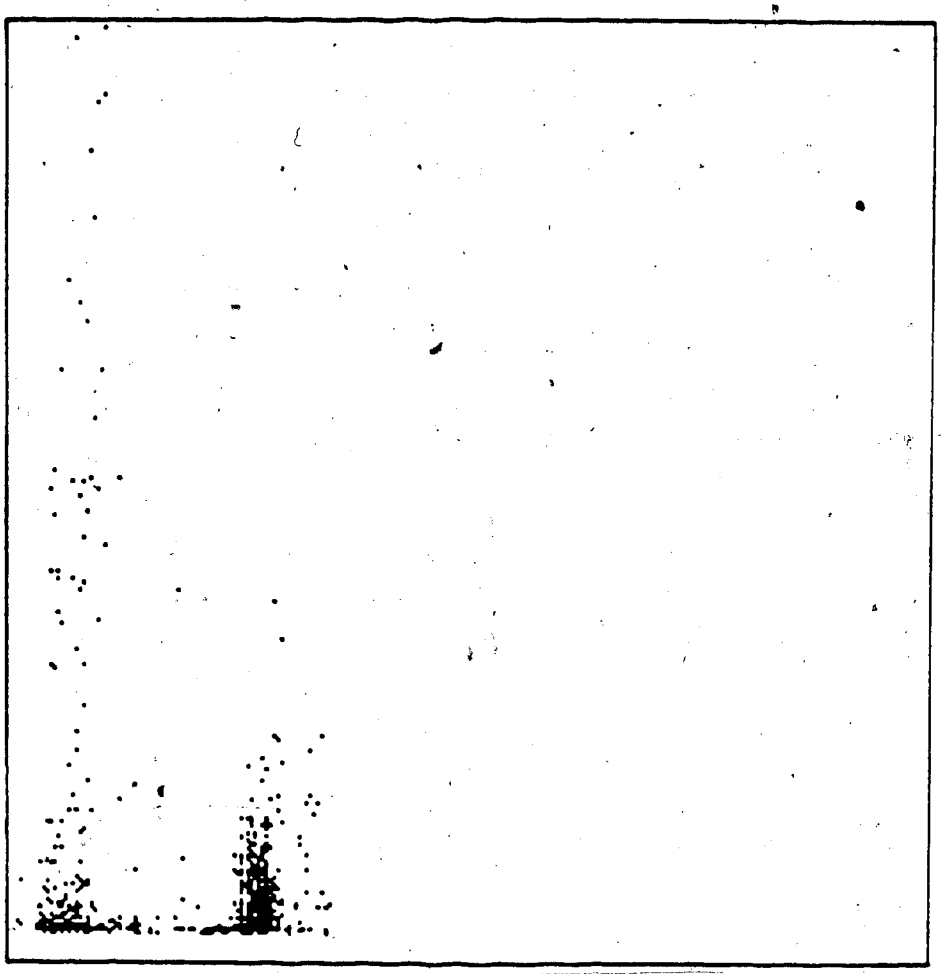

Figure 7-29 Vocalization Pattern Recorded for Rat #4 on Day 1.

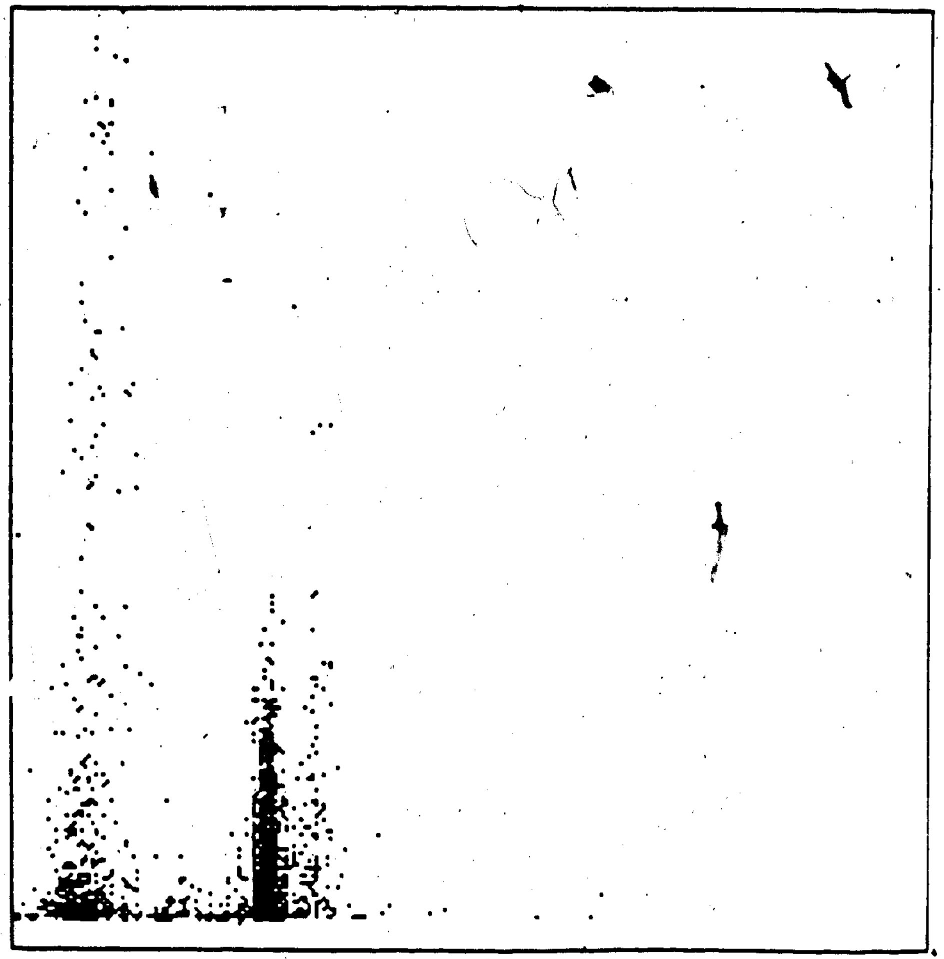

Figure 7-30 Vocalization Pattern Recorded for Rat #4 on Day 2.

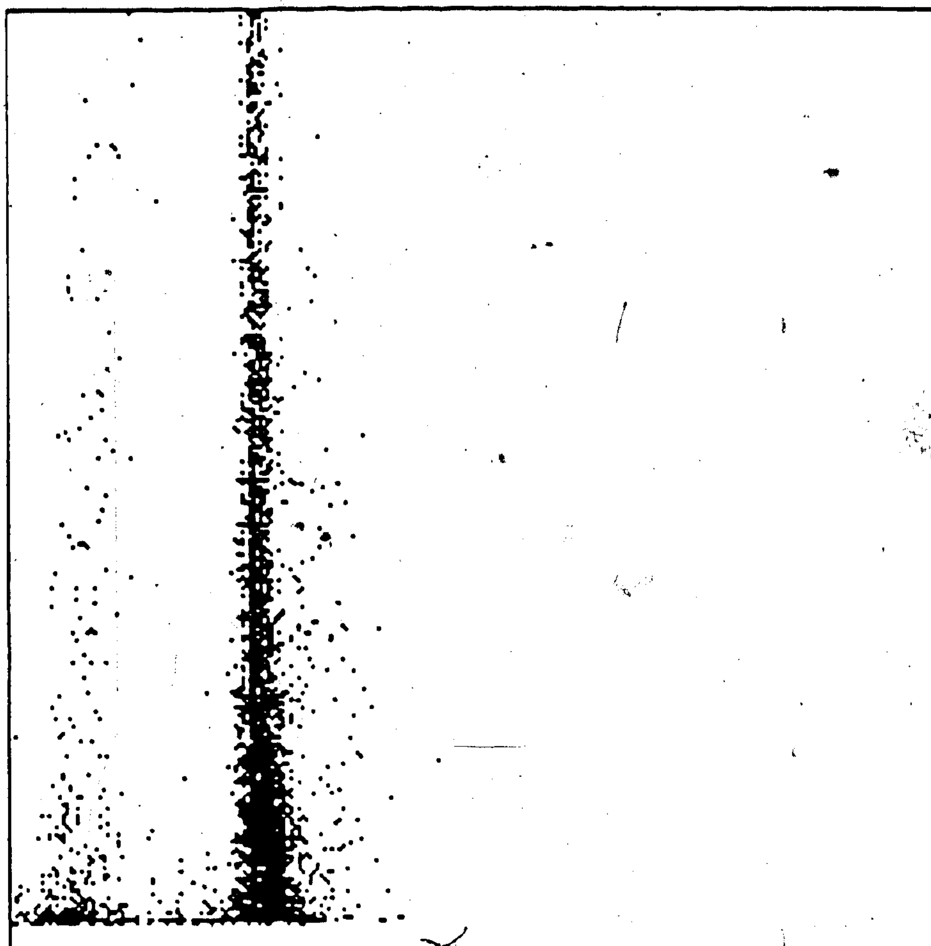

Figure 7-31 Vocalization Pattern Recorded for Rat #4 on Day 3.

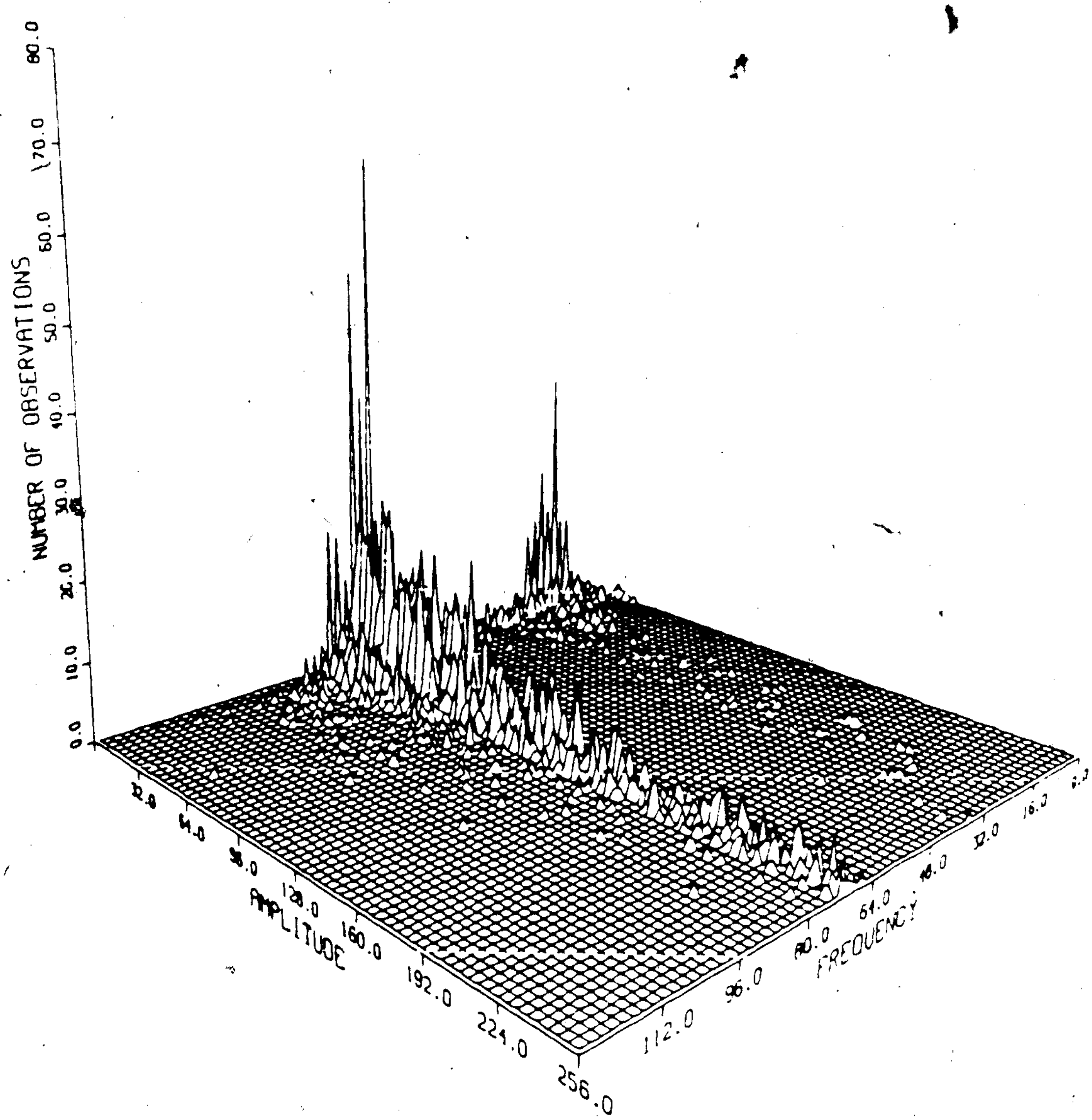

Figure 7-32 Surface plot of day 3 vocalizations for rat #4.

From the gray plots, it is evident that rats #2 and #4 were influenced by the stressor to a much greater extent than rats #1 and #3. This can also be seen in terms of the net vocalization time of each of these rats, listed in table 7-5.

Table 7-5 Stress Vocalization Times

Rat #	Training Period	# of stress call minutes	% of total recording time
#1	2 weeks	5.12 (2.90)[2]	1.73 (0.98)
#3	2 weeks	8.76	2.96
#2	1 week	15.82	19.78
#4	3 days[3]	8.28	20.75

When the frequency components of the datum sets were analyzed on a time interval basis, interesting results developed. Applying cluster analysis to the conglomeration of interval data recorded during the training periods resulted in the identification of two distinct vocalization patterns, labeled pattern I and pattern II. Pattern I represents sessions which contain few stress vocalizations. These vocalizations occurred sporadically throughout the recording session. Pattern II represents sessions which contain significant stress call levels. The second pattern can be further subdivided into two groups, IIa and IIb. Pattern IIa encompasses sessions in which the incidence of stress calls increases during the initial time intervals and then decreases during the final time intervals. Pattern IIb, on the otherhand, encompasses recording sessions in which the majority of stress calls occur during the final time intervals. Even though each rodent exhibited a particular vocalization pattern for the majority of its training session, the vocalization patterns may differ

[2]The values within the brackets do not include the session in which the light schedule was altered

[3]Rat #4 refused to run for the final two days of the training schedule

between the initial and final sessions (or between other sessions) due to the fact that the intensity of the training regime increased as the trials progressed.

It is highly indicative that the two major stress call patterns correspond to the severity with which the rats were affected by the training regime. Rats vocalizing according to pattern I perceived the running situation as being only mildly stressful. These rats appeared to have no difficulty adjusting to the various training regimes. Pattern II vocalizations, however, definitely elude to the fact that some of rats experienced significant levels of stress during the running trials. Stress calls present at the beginning of the pattern II vocalizations are most likely indicative of the initial interpretation of the stressful situation. Many of the rats with the pattern IIb vocalizations were not able to successfully deal with the stressful situation and thus spent a great deal of time on the shock plate. This inability to cope with the stressor was reflected by their poor performance on the treadmill; rats with pattern IIa vocalizations performed much more favourably on the treadmill.

It is interesting to note that the female rat reacted much more favourably to the stress situation then the males did. Stress vocalizations produced by the males usually resembled the pattern II vocalizations, whereas the female always vocalized according to pattern I. The males did not run nearly as well as their female counterpart and they usually appeared highly agitated during the trials. For this reason, a special trial was set up in which rat #10 was run for only one session at a speed of 30 m/min. for 120 minutes. Vocalizations were recorded during the first 5 minutes and the final 55 minutes of the session. Only 1 of the 7781 recorded calls contained a frequency component above 20 kHz, which substantiates the notion that the females were not severely affected by the exercise training regime. However, due to the relatively small number of females involved, it is difficult to assess the validity of the interpretation that the females were stressed to a lesser extent than their male counterparts. Figure 7–33 depicts the frequency information recorded from the two hour session. Figures 7–34 to 7–40 depict the frequency information recorded from other sessions.

Time Interval	From Interval 3.5	7.1	10.6	14.1	17.6	21.2	24.7	28.2	31.8	35.3	38.8	42.4	Total (%)
1	34 (13.6) (3.8)	145 (58.0) (3.3)	63 (25.2) (2.8)	7 (2.8) (4.2)	1 (0.4) (14.3)	0 (0.0) **	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	250 (3.2)
2	2 (0.5) (0.2)	57 (15.3) (1.3)	251 (67.3) (11.0)	62 (16.6) (37.3)	1 (0.3) (14.3)	0 (0.0) **	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	373 (4.8)
3	2 (0.9) (0.2)	42 (18.3) (0.9)	147 (63.9) (6.5)	36 (15.7) (21.7)	3 (1.3) (42.9)	0 (0.0) **	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	230 (3.0)
4	1 (2.8) (0.1)	14 (38.9) (0.3)	21 (58.3) (0.9)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	36 (0.5)
5	24 (7.5) (2.7)	200 (62.9) (4.5)	94 (29.6) (4.1)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	318 (4.1)
6	13 (3.1) (1.5)	246 (58.3) (5.5)	161 (38.2) (7.1)	2 (0.5) (1.2)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	422 (5.4)
7	7 (2.2) (0.8)	169 (52.6) (3.8)	139 (43.3) (6.1)	5 (1.6) (3.0)	1 (0.3) (14.3)	0 (0.0) **	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	321 (4.1)
8	10 (5.0) (1.1)	142 (71.0) (3.2)	48 (24.0) (2.1)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	200 (2.6)
9	241 (22.1) (26.8)	675 (62.0) (15.2)	166 (15.2) (7.3)	7 (0.6) (4.2)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	1089 (14.0)
10	0 ** (0.0)	0 ** (0.0)	0 ** (0.0)	0 ** (0.0)	0 ** (0.0)	0 ** **	0 ** (0.0)	0 ** **	0 ** **	0 ** **	0 ** **	0 ** **	0 (0.0)
11	26 (4.3) (2.9)	389 (64.8) (8.8)	180 (30.0) (7.9)	5 (0.8) (3.0)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	600 (7.7)
12	55 (5.7) (6.1)	585 (60.4) (13.2)	321 (33.1) (14.1)	8 (0.8) (4.8)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	969 (12.5)
13	27 (3.4) (3.0)	516 (65.1) (11.6)	247 (31.1) (10.8)	3 (0.4) (1.8)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	793 (10.2)
14	147 (16.2) (16.4)	566 (62.5) (12.8)	173 (19.1) (7.6)	18 (2.0) (10.8)	1 (0.1) (14.3)	0 (0.0) **	1 (0.1) (100.)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	906 (11.6)
15	1 (0.9) (0.1)	67 (59.8) (1.5)	44 (39.3) (1.9)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	112 (1.4)
16	38 (6.7) (4.2)	333 (58.3) (7.5)	189 (33.1) (8.3)	11 (1.9) (6.6)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	571 (7.3)
17	267 (45.2) (29.8)	287 (48.6) (6.5)	35 (5.9) (1.5)	2 (0.3) (1.2)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	591 (7.6)
Total	898 (11.5)	4433 (57.0)	2279 (29.3)	166 (2.1)	7 (0.1)	0 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	7781

Figure 7-33 Frequency Spectrum Recorded for Rat # 10

Day 1

Time Interval	Freq Interval 3.5	7.1	10.6	14.1	17.6	21.2	24.7	28.2	31.8	35.3	38.8	42.4	Total (%)
1	13 (20.0) (18.3)	41 (62.1) (11.6)	8 (12.1) (6.1)	1 (1.5) (5.6)	1 (1.5) (33.3)	0 (0.0) ..	0 (0.0) ..	0 (0.0) ..	0 (0.0) ..	0 (0.0) ..	0 (0.0) ..	0 (0.0) ..	66 (11.3)
2	14 (8.3) (19.7)	106 (63.1) (30.0)	38 (22.6) (28.8)	8 (4.8) (44.4)	2 (1.2) (66.7)	0 (0.0) ..	0 (0.0) ..	0 (0.0) ..	0 (0.0) ..	0 (0.0) ..	0 (0.0) ..	0 (0.0) ..	168 (28.1)
3	26 (15.5) (36.6)	102 (60.7) (28.9)	38 (22.6) (28.8)	2 (1.2) (11.1)	0 (0.0) (0.0)	0 (0.0) ..	0 (0.0) ..	0 (0.0) ..	0 (0.0) ..	0 (0.0) ..	0 (0.0) ..	0 (0.0) ..	168 (28.1)
4	13 (10.1) (18.3)	78 (60.5) (22.1)	34 (26.4) (25.8)	4 (3.1) (22.2)	0 (0.0) (0.0)	0 (0.0) ..	0 (0.0) ..	0 (0.0) ..	0 (0.0) ..	0 (0.0) ..	0 (0.0) ..	0 (0.0) ..	129 (22.4)
5	5 (10.6) (7.0)	26 (55.3) (7.4)	13 (27.7) (9.8)	3 (6.4) (16.7)	0 (0.0) (0.0)	0 (0.0) ..	0 (0.0) ..	0 (0.0) ..	0 (0.0) ..	0 (0.0) ..	0 (0.0) ..	0 (0.0) ..	47 (8.1)
Total	71 (12.3)	353 (61.2)	132 (22.9)	18 (3.1)	3 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	577

Day 10

Time Interval	Freq Interval 3.5	7.1	10.6	14.1	17.6	21.2	24.7	28.2	31.8	35.3	38.8	42.4	Total (%)
1	7 (9.7) (15.9)	31 (43.1) (2.5)	32 (44.4) (4.1)	2 (2.8) (3.3)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) ..	0 (0.0) ..	72 (1.6)
2	20 (7.6) (45.5)	123 (46.8) (9.7)	110 (41.8) (13.9)	8 (3.0) (13.3)	0 (0.0) (0.0)	2 (0.8) (5.7)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) ..	0 (0.0) ..	263 (6.0)
3	1 (0.4) (2.3)	141 (58.7) (11.2)	[illegible]7 (3[illegible]) (1[illegible])	11 (4.6) (18.3)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) ..	0 (0.0) ..	240 (5.4)
4	3 (1.2) (6.8)	153 (59.1) (12.1)	97 (37.5) (12.3)	6 (2.3) (10.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) ..	0 (0.0) ..	259 (5.9)
5	3 (1.0) (6.8)	178 (59.9) (14.1)	106 (35.7) (13.4)	7 (2.4) (11.7)	3 (1.0) (30.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) ..	0 (0.0) ..	297 (6.7)
6	2 (0.5) (4.5)	236 (58.7) (18.7)	151 (37.6) (19.1)	12 (3.0) (20.0)	1 (0.2) (10.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) ..	0 (0.0) ..	402 (9.1)
7	4 ([illegible]1) ([illegible]1)	233 (62.8) (18.5)	123 (33.2) (15.6)	9 (2.4) (15.0)	2 (0.5) (20.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) ..	0 (0.0) ..	371 (8.4)
8	1 (0.4) (2.3)	160 (66.9) (12.7)	77 (32.2) (9.8)	1 (0.4) (1.7)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) ..	0 (0.0) ..	239 (5.4)
9	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	15 (100.) (1.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) ..	0 (0.0) ..	15 (0.3)
10	2 (0.3) (4.5)	5 (0.8) (0.4)	5 (0.8) (0.6)	3 (0.5) (5.0)	2 (0.3) (20.0)	11 (1.8) (31.4)	226 (36.6) (34.0)	339 (54.9) (22.2)	23 (3.7) (92.0)	2 (0.3) (100.)	0 (0.0) ..	0 (0.0) ..	618 (14.0)
11	1 (0.1) (2.3)	1 (0.1) (0.1)	0 (0.0) (0.0)	0 (0.0) (0.0)	2 (0.2) (20.0)	15 (1.2) (42.9)	283 (22.1) (42.6)	978 (76.3) (64.1)	2 (0.2) (8.0)	0 (0.0) (0.0)	0 (0.0) ..	0 (0.0) ..	1282 (29.0)
12	0 (0.0) (0.0)	1 (0.3) (0.1)	1 (0.3) (0.1)	1 (0.3) (1.7)	0 (0.0) (0.0)	7 (1.9) (20.0)	155 (43.2) (23.3)	194 (54.0) (12.7)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) ..	0 (0.0) ..	359 (8.1)
Total	44 (1.0)	1262 (28.6)	788 (17.8)	60 (1.4)	10 (0.2)	35 (0.8)	664 (15.0)	1526 (34.5)	25 (0.6)	2 (0.0)	0 (0.0)	0 (0.0)	4417

Figure 7-34 Frequency Spectrum Recorded for Rat #1

Day 1

Time Interval	Freq Interval 3.5	7.1	10.6	14.1	17.6	21.2	24.7	28.2	31.8	35.3	38.8	42.4	Total (%)
1	4 (2.6) (5.6)	80 (52.3) (29.4)	67 (43.8) (33.7)	2 (1.3) (25.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	153 (27.7)
2	67 (16.8) (94.4)	192 (48.1) (70.6)	132 (33.1) (66.3)	6 (1.5) (75.0)	1 (0.3) (100)	1 (0.3) (100)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	399 (72.3)
Total	71 (12.9)	272 (49.3)	199 (36.1)	8 (1.4)	1 (0.2)	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	552

Day 3

Time Interval	Freq Interval 3.5	7.1	10.6	14.1	17.6	21.2	24.7	28.2	31.8	35.3	38.8	42.4	Total (%)
1	26 (18.7) (11.9)	60 (43.2) (6.1)	49 (35.3) (7.8)	4 (2.9) (6.2)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	139 (7.3)
2	54 (12.5) (24.8)	230 (53.4) (23.5)	133 (30.9) (21.2)	13 (3.0) (20.3)	1 (0.2) (16.7)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	431 (22.8)
3	62 (14.0) (28.4)	240 (54.2) (24.6)	132 (29.8) (21.1)	9 (2.0) (14.1)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	443 (23.4)
4	53 (9.9) (24.3)	273 (50.8) (27.9)	190 (35.4) (30.3)	19 (3.5) (2[illegible]	2 (0.4) (33.3)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	537 (28.4)
5	23 (6.7) (10.6)	174 (50.9) (17.8)	123 (36.0) (19.6)	19 (5.6) (29.7)	3 (0.9) (50.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	342 (18.1)
Total	218 (11.5)	977 (51.6)	627 (33.1)	64 (3.4)	6 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1892

Day 10

Time Interval	Freq Interval 3.5	7.1	10.6	14.1	17.6	21.2	24.7	28.2	31.8	35.3	38.8	42.4	Total (%)
1	3 (2.5) (5.1)	63 (51.6) (3.4)	47 (38.5) (2.7)	7 (5.7) (1.3)	2 (1.6) (1.1)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	122 (1.7)
2	10 (2.0) (16.9)	303 (59.3) (16.2)	173 (33.9) (10.0)	24 (4.7) (4.5)	1 (0.2) (0.5)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	511 (7.0)
3	2 (0.5) (3.4)	218 (57.1) (11.7)	160 (41.9) (9.3)	2 (0.5) (0.4)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	382 (5.2)
4	4 (0.6) (6.8)	326 (45.5) (17.4)	270 (37.7) (15.7)	90 (12.6) (16.9)	23 (3.2) (12.5)	4 (0.6) (5.8)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	717 (9.8)
5	11 (1.8) (18.6)	262 (44.0) (14.0)	212 (35.6) (12.3)	81 (13.6) (15.3)	29 (4.9) (15.8)	1 (0.2) (1.4)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	596 (8.1)
6	4 (0.6) (6.8)	240 (36.4) (12.8)	305 (46.2) (17.7)	77 (11.7) (14.5)	30 (4.5) (16.3)	4 (0.6) (5.8)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	660 (9.0)
7	2 (0.2) (3.4)	307 (36.0) (16.4)	355 (41.6) (20.6)	133 (15.6) (25.0)	44 (5.2) (23.9)	11 (1.3) (15.9)	1 (0.1) (0.0)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	853 (11.7)
8	3 (0.4) (5.1)	133 (16.8) (7.1)	188 (23.7) (10.9)	109 (13.8) (20.5)	46 (5.8) (25.0)	10 (1.3) (14.5)	295 (37.2) (12.1)	8 (1.0) (1.8)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	792 (10.8)
9	13 (2.9) (22.0)	15 (3.3) (0.8)	11 (2.4) (0.6)	5 (1.1) (0.9)	4 (0.9) (2.2)	11 (2.4) (15.9)	326 (72.6) (13.4)	64 (14.3) (14.4)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	449 (6.1)
10	5 (0.5) (8.5)	0 (0.0) (0.0)	0 (0.0) (0.0)	1 (0.1) (0.2)	4 (0.4) (2.2)	16 (1.6) (23.2)	900 (89.8) (36.9)	76 (7.6) (17.2)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	1002 (13.7)
11	1 (0.1) (1.7)	0 (0.0) (0.0)	1 (0.1) (0.1)	1 (0.1) (0.2)	1 (0.1) (0.5)	12 (1.0) (17.4)	893 (74.9) (36.6)	283 (23.7) (63.9)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	1192 (16.3)
12	1 (2.4) (1.7)	2 (4.8) (0.1)	0 (0.0) (0.0)	1 (2.4) (0.2)	0 (0.0) (0.0)	0 (0.0) (0.0)	26 (61.9) (1.1)	12 (28.6) (2.7)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	42 (0.6)
Total	59 (0.8)	1868 (25.5)	1722 (23.5)	531 (7.3)	184 (2.5)	69 (0.9)	2441 (33.4)	443 (6.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	7318

Figure 7–35 Frequency Spectrum Recorded for Rat #3

Day 1

Freq Interval

Time Interval	3.5	7.1	10.6	14.1	17.6	21.2	24.7	28.2	31.8	35.3	38.8	42.4	Total (%)
1	33 (1.7) (100.)	17 (0.9) (100.)	2 (0.1) (100.)	2 (0.1) (100)	6 (0.3) (100)	10 (0.5) (100)	1787 (91.3) (100)	77 (3.9) (100)	8 (0.4) (100)	8 (0.4) (100)	3 (0.2) (100)	5 (0.3) (100)	1958 (100.0)
Total	33 (1.7)	17 (0.9)	2 (0.1)	2 (0.1)	6 (0.3)	10 (0.5)	1787 (91.3)	77 (3.9)	8 (0.4)	8 (0.4)	3 (0.2)	5 (0.3)	1958

Day 2

Freq Interval

Time Interval	3.5	7.1	10.6	14.1	17.6	21.2	24.7	28.2	31.8	35.3	38.8	42.4	Total (%)
1	13 (0.9) (41.9)	1 (0.1) (100)	1 (0.1) (100)	1 (0.1) (100)	2 (0.1) (50.0)	130 (8.8) (63.1)	1328 (89.8) (50.5)	3 (0.2) (75.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	1479 (51.4)
2	18 (1.3) (58.1)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	2 (0.1) (50.0)	76 (5.4) (36.9)	1301 (93.1) (49.5)	1 (0.1) (25.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	1398 (48.6)
Total	31 (1.1)	1 (0.0)	1 (0.0)	1 (0.0)	4 (0.1)	206 (7.2)	2629 (91.4)	4 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2877

Day 3

Freq Interval

Time Interval	3.5	7.1	10.6	14.1	17.6	21.2	24.7	28.2	31.8	35.3	38.8	42.4	Total (%)
1	12 (2.0) (70.6)	2 (0.3) (50.0)	2 (0.3) (50.0)	2 (0.3) (50.0)	3 (0.5) (21.4)	2 (0.3) (2.2)	566 (93.1) (26.6)	19 (3.1) (73.1)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	607 (26.6)
2	2 (0.7) (11.8)	1 (0.3) (25.0)	1 (0.3) (25.0)	0 (0.0) (0.0)	2 (0.7) (14.3)	22 (7.4) (24.7)	267 (89.3) (12.6)	4 (1.3) (15.4)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	299 (13.1)
3	3 (0.2) (17.6)	1 (0.1) (25.0)	1 (0.1) (25.0)	2 (0.1) (50.0)	9 (0.7) (64.3)	65 (4.7) (73.0)	1293 (93.9) (60.8)	3 (0.2) (11.5)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	1377 (60.3)
Total	17 (0.7)	4 (0.2)	4 (0.2)	4 (0.2)	14 (0.6)	89 (3.9)	2125 (93.1)	26 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2283

Day 4

Freq Interval

Time Interval	3.5	7.1	10.6	14.1	17.6	21.2	24.7	28.2	31.8	35.3	38.8	42.4	Total (%)
1	15 (2.3) (50.0)	357 (54.9) (91.5)	241 (37.1) (92.0)	22 (3.4) (78.6)	5 (0.8) (45.5)	0 (0.0) (0.0)	0 (0.0) (0.0)	8 (1.2) (24.2)	2 (0.3) (66.7)	0 (0.0) **	0 (0.0) **	0 (0.0) **	650 (28.3)
2	10 (0.8) (33.3)	33 (2.5) (8.5)	20 (1.5) (7.6)	5 (0.4) (17.9)	3 (0.2) (27.3)	93 (7.1) (78.2)	1116 (85.5) (78.7)	25 (1.9) (75.8)	1 (0.1) (33.3)	0 (0.0) **	0 (0.0) **	0 (0.0) **	1306 (56.9)
3	5 (1.5) (16.7)	0 (0.0) (0.0)	1 (0.3) (0.4)	1 (0.3) (3.6)	3 (0.9) (27.3)	26 (7.7) (21.8)	302 (89.3) (21.3)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	338 (14.7)
Total	30 (1.3)	390 (17.0)	262 (11.4)	28 (1.2)	11 (0.5)	119 (5.2)	1418 (61.8)	33 (1.4)	3 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	2294

Figure 7-36 Frequency Spectrum Recorded for Rat #2

Day 1

Time Interval \ Freq Interval	3.5	7.1	10.6	14.1	17.6	21.2	24.7	28.2	31.8	35.3	38.8	42.4	Total (%)
1	6 (2.4) (75.0)	116 (45.8) (96.7)	96 (37.9) (94.1)	16 (6.3) (80.0)	3 (1.2) (23.1)	2 (0.8) (5.4)	7 (2.8) (1.5)	5 (2.0) (1.3)	2 (0.8) (8.3)	0 (0.0) **	0 (0.0) **	0 (0.0) **	253 (21.6)
2	2 (0.2) (25.0)	4 (0.4) (3.3)	6 (0.7) (5.9)	4 (0.4) (20.0)	10 (1.1) (76.9)	35 (3.8) (94.6)	459 (49.9) (98.5)	378 (41.1) (98.7)	22 (2.4) (91.7)	0 (0.0) **	0 (0.0) **	0 (0.0) **	920 (78.4)
Total	8 (0.7)	120 (10.2)	102 (8.7)	20 (1.7)	13 (1.1)	37 (3.2)	466 (39.7)	383 (32.7)	24 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)	1173

Day 2

Time Interval \ Freq Interval	3.5	7.1	10.6	14.1	17.6	21.2	24.7	28.2	31.8	35.3	38.8	42.4	Total (%)
1	14 (4.6) (36.8)	93 (30.3) (31.6)	61 (19.9) (17.8)	12 (3.9) (16.9)	17 (5.5) (22.1)	14 (4.6) (12.5)	19 (6.2) (2.7)	54 (17.6) (3.9)	22 (7.2) (17.3)	1 (0.3) (11.1)	0 (0.0) (0.0)	0 (0.0) (0.0)	307 (9.7)
2	9 (1.6) (23.7)	133 (24.3) (45.2)	177 (32.4) (51.8)	31 (5.7) (43.7)	14 (2.6) (18.2)	7 (1.3) (6.2)	21 (3.8) (3.0)	39 (7.1) (2.8)	103 (18.8) (81.1)	8 (1.5) (88.9)	3 (0.5) (100)	2 (0.4) (100)	547 (17.4)
3	9 (5.2) (23.7)	57 (33.1) (19.4)	91 (52.9) (26.6)	14 (8.1) (19.7)	1 (0.6) (1.3)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	172 (5.5)
4	4 (0.3) (10.5)	9 (0.7) (3.1)	10 (0.8) (2.9)	10 (0.8) (14.1)	26 (2.0) (33.8)	79 (5.9) (70.5)	458 (34.5) (64.9)	730 (55.0) (53.3)	2 (0.2) (1.6)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	1328 (42.2)
5	2 (0.3) (5.3)	2 (0.2) (0.7)	3 (0.4) (0.9)	4 (0.5) (5.6)	19 (2.4) (24.7)	12 (1.5) (10.7)	208 (26.1) (29.5)	546 (68.6) (39.9)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	796 (25.3)
Total	38 (1.2)	294 (9.3)	342 (10.9)	71 (2.3)	77 (2.4)	112 (3.6)	706 (22.4)	1369 (43.5)	127 (4.0)	9 (0.3)	3 (0.1)	2 (0.1)	3150

Day 3

Time Interval \ Freq Interval	3.5	7.1	10.6	14.1	17.6	21.2	24.7	28.2	31.8	35.3	38.8	42.4	Total (%)
1	0 ** (0.0)	0 ** (0.0)	0 ** (0.0)	0 ** (0.0)	0 ** (0.0)	0 ** (0.0)	0 ** (0.0)	0 ** (0.0)	0 ** (0.0)	0 ** (0.0)	0 ** (0.0)	0 ** (0.0)	0 (0.0)
2	6 (0.7) (13.0)	9 (1.1) (3.2)	7 (0.8) (3.3)	4 (0.5) (6.3)	5 (0.6) (11.4)	16 (1.9) (25.8)	540 (64.7) (23.3)	232 (27.8) (9.4)	6 (0.7) (4.0)	4 (0.5) (12.1)	5 (0.6) (55.6)	0 (0.0) (0.0)	834 (14.7)
3	2 (0.3) (4.3)	1 (0.2) (0.4)	2 (0.3) (0.9)	2 (0.3) (3.2)	2 (0.3) (4.5)	4 (0.6) (6.5)	334 (50.6) (14.4)	311 (47.1) (12.6)	2 (0.3) (1.3)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	660 (11.6)
4	11 (0.7) (23.9)	2 (0.1) (0.7)	4 (0.2) (1.9)	2 (0.1) (3.2)	5 (0.3) (11.4)	18 (1.1) (29.0)	773 (46.9) (33.4)	778 (47.2) (31.4)	54 (3.3) (36.2)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	1647 (29.0)
5	1 (0.6) (2.2)	0 (0.0) (0.0)	1 (0.6) (0.5)	6 (3.4) (9.5)	3 (1.7) (6.8)	1 (0.6) (1.6)	6 (3.4) (0.3)	143 (81.7) (5.8)	14 (8.0) (9.4)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	175 (3.1)
6	24 (1.1) (52.2)	265 (12.5) (95.7)	195 (9.2) (91.5)	47 (2.2) (74.6)	27 (1.3) (61.4)	22 (1.0) (35.5)	646 (30.5) (27.9)	786 (37.1) (31.8)	73 (3.4) (49.0)	29 (1.4) (87.9)	4 (0.2) (44.4)	1 (0.0) (100)	2119 (37.3)
7	2 (0.8) (4.3)	0 (0.0) (0.0)	4 (1.6) (1.9)	2 (0.8) (3.2)	2 (0.8) (4.5)	1 (0.4) (1.6)	16 (6.3) (0.7)	225 (89.3) (9.1)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	252 (4.4)
Total	46 (0.8)	277 (4.9)	213 (3.7)	63 (1.1)	44 (0.8)	62 (1.1)	2315 (40.7)	2475 (43.5)	149 (2.6)	33 (0.6)	9 (0.2)	1 (0.0)	5687

Figure 7-37 Frequency Spectrum Recorded for Rat #4

Day 2

Time Interval	Freq Interval 3.5	7.1	10.6	14.1	17.6	21.2	24.7	28.2	31.8	35.3	38.8	42.4	Total (%)
1	810 (75.6) (47.4)	51 (4.8) (15.7)	13 (1.2) (3.8)	7 (0.7) (4.2)	7 (0.7) (10.3)	4 (0.4) (40.0)	1 (0.1) (8.3)	138 (12.9) (52.9)	27 (2.5) (38.0)	7 (0.7) (77.8)	6 (0.6) (100.)	0 (0.0) **	1071 (35.9)
2	76 (67.3) (4.4)	10 (8.8) (3.1)	4 (3.5) (1.2)	1 (0.8) (0.6)	1 (0.9) (1.5)	0 (0.0) (0.0)	2 (1.8) (16.7)	19 (16.8) (7.3)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	113 (3.8)
3	1 (1.2) (0.1)	16 (19.3) (4.9)	35 (42.2) (10.1)	23 (27.7) (13.9)	8 (9.6) (11.8)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	83 (2.8)
4	508 (46.5) (29.8)	134 (12.2) (41.2)	166 (15.2) (48.1)	87 (7.9) (52.7)	35 (3.2) (51.5)	5 (0.5) (50.0)	9 (0.8) (75.0)	104 (9.5) (39.8)	44 (4.0) (62.0)	2 (0.2) (22.2)	0 (0.0) (0.0)	0 (0.0) **	1095 (36.7)
5	30 (10.4) (1.8)	97 (33.7) (28.8)	102 (35.4) (29.6)	41 (14.2) (24.8)	17 (5.9) (25.0)	1 (0.3) (10.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	288 (9.7)
6	284 (85.5) (16.6)	17 (5.1) (5.2)	25 (7.5) (7.2)	6 (1.8) (3.6)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	332 (11.1)
Total	1710 (57.3)	325 (10.9)	345 (11.6)	165 (5.5)	68 (2.3)	10 (0.3)	12 (0.4)	261 (8.8)	71 (2.4)	9 (0.3)	6 (0.2)	0 (0.0)	2982

Day 3

Time Interval	Freq Interval 3.5	7.1	10.6	14.1	17.6	21.2	24.7	28.2	31.8	35.3	38.8	42.4	Total (%)
1	0 ** (0.0)	0 ** (0.0)	0 ** (0.0)	0 ** (0.0)	0 ** (0.0)	0 ** (0.0)	0 ** (0.0)	0 ** **	0 ** **	0 ** **	0 ** **	0 ** **	0 (0.0)
2	58 (6.7) (27.6)	447 (51.5) (27.3)	300 (34.6) (23.5)	48 (5.6) (19.6)	14 (1.6) (18.9)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	868 (25.1)
3	4 (0.8) (1.9)	141 (27.5) (8.6)	258 (50.4) (20.3)	79 (15.4) (31.6)	18 (3.5) (24.3)	10 (2.0) (100.)	1 (0.2) (100.)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	512 (14.8)
4	51 (7.1) (24.3)	361 (50.1) (22.0)	229 (31.8) (18.0)	50 (6.9) (20.0)	30 (4.2) (40.5)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	721 (20.9)
5	37 (4.8) (17.6)	298 (38.9) (18.2)	368 (48.0) (28.9)	56 (7.3) (22.4)	8 (1.0) (10.8)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	767 (22.2)
6	40 (8.6) (19.0)	309 (66.5) (18.9)	97 (20.9) (7.6)	15 (3.2) (6.0)	4 (0.9) (5.4)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	465 (13.5)
7	20 (16.1) (9.5)	82 (66.1) (5.0)	21 (16.9) (1.6)	1 (0.8) (0.4)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	124 (3.6)
Total	210 (6.1)	1638 (47.4)	1274 (36.9)	250 (7.2)	74 (2.1)	10 (0.3)	1 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3457

Day 4

Time Interval	Freq Interval 3.5	7.1	10.6	14.1	17.6	21.2	24.7	28.2	31.8	35.3	38.8	42.4	Total (%)
1	12 (3.5) (14.6)	154 (44.9) (10.3)	142 (41.4) (6.7)	28 (8.2) (12.4)	7 (2.0) (17.9)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	343 (8.7)
2	16 (1.8) (19.5)	382 (43.6) (25.6)	435 (49.6) (20.6)	37 (4.2) (16.4)	7 (0.8) (17.9)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	877 (22.2)
3	15 (1.6) (18.3)	373 (39.4) (25.0)	508 (53.6) (24.0)	47 (5.0) (20.9)	4 (0.4) (10.3)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	947 (23.9)
4	13 (1.5) (15.9)	293 (34.2) (19.6)	510 (59.5) (24.1)	37 (4.3) (16.4)	4 (0.5) (10.3)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	857 (21.7)
5	13 (1.9) (15.9)	230 (33.9) (15.4)	389 (57.4) (18.4)	43 (6.3) (19.1)	3 (0.4) (7.7)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	678 (17.1)
6	1 (1.1) (1.2)	21 (23.9) (1.4)	59 (67.0) (2.8)	7 (8.0) (3.1)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	88 (2.2)
7	12 (7.2) (14.6)	41 (24.7) (2.7)	70 (42.2) (3.3)	26 (15.7) (11.6)	14 (8.4) (35.9)	2 (1.2) (100)	1 (0.6) (100.)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	166 (4.2)
Total	82 (2.1)	1494 (37.8)	2113 (53.4)	225 (5.7)	39 (1.0)	2 (0.1)	1 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3954

Figure 7-38 Frequency Spectrum Recorded for Rat #6

Day 1

Freq Interval

Time Interval	3.5	7.1	10.6	14.1	17.6	21.2	24.7	28.2	31.8	35.3	38.8	42.4	Total (%)
1	41	265	88	4	0	0	0	0	0	0	0	0	398
	(10.3)	(66.6)	(22.1)	(1.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(38.0)
	(61.2)	(38.7)	(31.4)	(25.0)	**	**	**	**	**	**	**	**	
2	26	420	192	12	0	0	0	0	0	0	0	0	650
	(4.0)	(64.6)	(29.5)	(1.8)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(62.0)
	(38.8)	(61.3)	(68.6)	(75.0)	**	**	**	**	**	**	**	**	
Total	67	685	280	16	0	0	0	0	0	0	0	0	1048
	(6.4)	(65.4)	(26.7)	(1.5)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	

Day 3

Freq Interval

Time Interval	3.5	7.1	10.6	14.1	17.6	21.2	24.7	28.2	31.8	35.3	38.8	42.4	Total (%)
1	0	0	0	0	0	0	0	0	0	0	0	0	0
	**	**	**	**	**	**	**	**	**	**	**	**	(0.0)
	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	**	**	**	**	**	
2	69	128	112	33	16	1	3	0	0	0	0	0	362
	(19.1)	(35.4)	(30.9)	(9.1)	(4.4)	(0.3)	(0.8)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(16.4)
	(7.1)	(15.3)	(36.0)	(45.8)	(80.0)	(50.0)	(100.0)	**	**	**	**	**	
3	181	228	88	18	3	0	0	0	0	0	0	0	518
	(34.9)	(44.0)	(17.0)	(3.5)	(0.6)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(23.4)
	(18.7)	(27.3)	(28.3)	(25.0)	(15.0)	(0.0)	(0.0)	**	**	**	**	**	
4	423	400	85	13	1	1	0	0	0	0	0	0	923
	(45.8)	(43.3)	(9.2)	(1.4)	(0.1)	(0.1)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(41.8)
	(43.7)	(48.0)	(27.3)	(18.1)	(5.0)	(50.0)	(0.0)	**	**	**	**	**	
5	295	78	26	8	0	0	0	0	0	0	0	0	407
	(72.5)	(19.2)	(6.4)	(2.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(18.4)
	(30.5)	(9.4)	(8.4)	(11.1)	(0.0)	(0.0)	(0.0)	**	**	**	**	**	
Total	968	834	311	72	20	2	3	0	0	0	0	0	2210
	(43.8)	(37.7)	(14.1)	(3.3)	(0.9)	(0.1)	(0.1)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	

Day 5

Freq Interval

Time Interval	3.5	7.1	10.6	14.1	17.6	21.2	24.7	28.2	31.8	35.3	38.8	42.4	Total (%)
1	3	1	95	28	4	0	0	0	0	0	0	0	131
	(2.3)	(0.8)	(72.5)	(21.4)	(3.1)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(4.8)
	(9.4)	(0.4)	(23.7)	(24.1)	(20.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	**	
2	18	185	236	59	7	1	0	0	0	0	0	0	506
	(3.6)	(36.6)	(46.6)	(11.7)	(1.4)	(0.2)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(18.4)
	(56.2)	(82.6)	(59.0)	(50.9)	(35.0)	(4.2)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	**	
3	4	9	2	8	1	7	477	501	21	7	0	0	1037
	(0.4)	(0.9)	(0.2)	(0.8)	(0.1)	(0.7)	(46.0)	(48.3)	(2.0)	(0.7)	(0.0)	(0.0)	(37.8)
	(12.5)	(4.0)	(0.5)	(6.9)	(5.0)	(29.2)	(65.5)	(45.1)	(29.2)	(46.7)	(0.0)	**	
4	4	9	30	10	7	16	250	609	42	0	0	0	977
	(0.4)	(0.9)	(3.1)	(1.0)	(0.7)	(1.6)	(25.6)	(62.3)	(4.3)	(0.0)	(0.0)	(0.0)	(35.6)
	(12.5)	(4.0)	(7.5)	(8.6)	(35.0)	(66.7)	(34.3)	(54.9)	(58.3)	(0.0)	(0.0)	**	
5	3	20	37	11	1	0	1	0	9	8	3	0	93
	(3.2)	(21.5)	(39.8)	(11.8)	(1.1)	(0.0)	(1.1)	(0.0)	(9.7)	(8.6)	(3.2)	(0.0)	(3.4)
	(9.4)	(8.9)	(9.2)	(9.5)	(5.0)	(0.0)	(0.1)	(0.0)	(12.5)	(53.3)	(100.0)	**	
Total	32	224	400	116	20	24	728	1110	72	15	3	0	2744
	(1.2)	(8.2)	(14.6)	(4.2)	(0.7)	(0.9)	(26.5)	(40.5)	(2.6)	(0.5)	(0.1)	(0.0)	

Figure 7-39 Frequency Spectrum Recorded for Rat #7

Day 1

Time Interval	Freq Interval 3.5	7.1	10.6	14.1	17.6	21.2	24.7	28.2	31.8	35.3	38.8	42.4	Total (%)
1	126 (10.1) (40.8)	747 (59.9) (38.9)	361 (28.9) (34.1)	13 (1.0) (30.2)	1 (0.1) (25.0)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	1248 (37.4)
2	133 (12.7) (43.0)	618 (59.0) (32.2)	288 (27.5) (27.2)	9 (0.9) (20.9)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	1048 (31.4)
3	50 (4.8) (16.2)	555 (53.2) (28.9)	411 (39.4) (38.8)	21 (2.0) (48.8)	3 (0.3) (75.0)	3 (0.3) (100)	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	0 (0.0) **	1043 (31.2)
Total	309 (9.3)	1920 (57.5)	1060 (31.7)	43 (1.3)	4 (0.1)	3 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3339

Day 3

Time Interval	Freq Interval 3.5	7.1	10.6	14.1	17.6	21.2	24.7	28.2	31.8	35.3	38.8	42.4	Total (%)
1	72 (51.1) (24.7)	48 (34.0) (1.9)	19 (13.5) (1.3)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	1 (0.7) (16.7)	0 (0.0) (0.0)	1 (0.7) (33.3)	0 (0.0) (0.0)	0 (0.0) **	141 (3.2)
2	12 (1.7) (4.1)	368 (50.6) (14.8)	322 (44.3) (21.8)	18 (2.5) (13.0)	1 (0.1) (7.7)	1 (0.1) (20.0)	2 (0.3) (40.0)	2 (0.3) (33.3)	1 (0.1) (25.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	727 (16.4)
3	26 (4.0) (8.9)	317 (48.5) (12.8)	287 (43.9) (19.5)	22 (3.4) (15.9)	0 (0.0) (0.0)	0 (0.0) (0.0)	1 (0.2) (20.0)	1 (0.2) (16.7)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	654 (14.8)
4	65 (3.8) (22.3)	1001 (58.2) (40.3)	598 (34.8) (40.5)	35 (2.0) (25.4)	7 (0.4) (53.8)	4 (0.2) (80.0)	2 (0.1) (40.0)	2 (0.1) (33.3)	3 (0.2) (75.0)	2 (0.1) (66.7)	1 (0.1) (100)	0 (0.0) **	1720 (38.9)
5	21 (12.4) (7.2)	99 (58.6) (4.0)	35 (20.7) (2.4)	13 (7.7) (9.4)	1 (0.6) (7.7)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	169 (3.8)
6	96 (9.5) (32.9)	648 (64.0) (26.1)	214 (21.1) (14.5)	50 (4.9) (36.2)	4 (0.4) (30.8)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	1012 (22.9)
Total	292 (6.6)	2481 (56.1)	1475 (33.3)	138 (3.1)	13 (0.3)	5 (0.1)	5 (0.1)	6 (0.1)	4 (0.1)	3 (0.1)	1 (0.0)	0 (0.0)	4423

Day 5

Time Interval	Freq Interval 3.5	7.1	10.6	14.1	17.6	21.2	24.7	28.2	31.8	35.3	38.8	42.4	Total (%)
1	990 (91.6) (38.9)	66 (6.1) (15.4)	20 (1.9) (27.4)	3 (0.3) (11.1)	0 (0.0) (0.0)	2 (0.2) (66.7)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	1081 (34.1)
2	925 (91.9) (36.4)	52 (5.2) (12.1)	20 (2.0) (27.4)	8 (0.8) (29.6)	1 (0.1) (12.5)	0 (0.0) (0.0)	1 (0.1) (9.1)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	1007 (31.8)
3	56 (91.8) (2.2)	3 (4.9) (0.7)	0 (0.0) (0.0)	2 (3.3) (7.4)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	61 (1.9)
4	327 (86.7) (12.9)	36 (9.5) (8.4)	9 (2.4) (12.3)	5 (1.3) (18.5)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	377 (11.9)
5	0 ** (0.0)	0 ** (0.0)	0 ** (0.0)	0 ** (0.0)	0 ** (0.0)	0 ** (0.0)	0 ** (0.0)	0 ** (0.0)	0 ** (0.0)	0 ** (0.0)	0 ** **	0 ** **	0 (0.0)
6	0 (0.0) (0.0)	1 (25.0) (0.2)	3 (75.0) (4.1)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	4 (0.1)
7	3 (8.3) (0.1)	17 (47.2) (4.0)	13 (36.1) (17.8)	1 (2.8) (3.7)	2 (5.6) (25.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) (0.0)	0 (0.0) **	0 (0.0) **	36 (1.1)
8	241 (39.8) (9.5)	253 (41.8) (59.1)	8 (1.3) (11.0)	8 (1.3) (29.6)	5 (0.8) (62.5)	1 (0.2) (33.3)	10 (1.7) (90.9)	38 (6.3) (100)	35 (5.8) (100)	6 (1.0) (100)	0 (0.0) **	0 (0.0) **	605 (19.1)
Total	2542 (80.2)	428 (13.5)	73 (2.3)	27 (0.9)	8 (0.3)	3 (0.1)	11 (0.3)	38 (1.2)	35 (1.1)	6 (0.2)	0 (0.0)	0 (0.0)	3171

Figure 7-40 Frequency Spectrum Recorded for Rat #9

2. Swimming Trials

Figures 7-41 to 7-44 illustrate the vocalizations recorded during the swimming trials. Judging from the relatively small number of stress vocalizations present in the figures, it appears that swimming does not elicit nearly the stress reaction that running does. In fact, no stress calls were even recorded during trial #1. A partial explanation for the lack of stress call content may stem from the fact that some of the rats developed a drownproofing sequence shortly after the trials began. This sequence required little energy on the part of the rat and hence did not accurately reflect a swimming situation.

Swimming more than one rat at a time provided a solution to eliminating the drownproofing sequence. Stress calls recorded during trials in which two rats were swimming at the same time (trials #3 and #4) resembled pattern I vocalizations. The vocalizations were few in number and occured sporadically throughout the recording session.

As expected, loading greatly increases the stress call content. Data recorded during trial #2 contained a significant number of stress vocalizations. These vocalizations occurred in large groups towards the latter half of the recording session. However, the number of stress vocalizations recorded during this trial is small compared to the number recorded from stressed rats during the running trials.

Under mildly turbulent water conditions, very few stress calls were recorded. When the water turbulence was increased, the extraneous noise generated by the turbulent water was of significant intensity to mask the stress vocalizations. Thus, even though it is very likely that the number of stress vocalizations would increase with increased turbulence, no concrete conclusions can be stated.

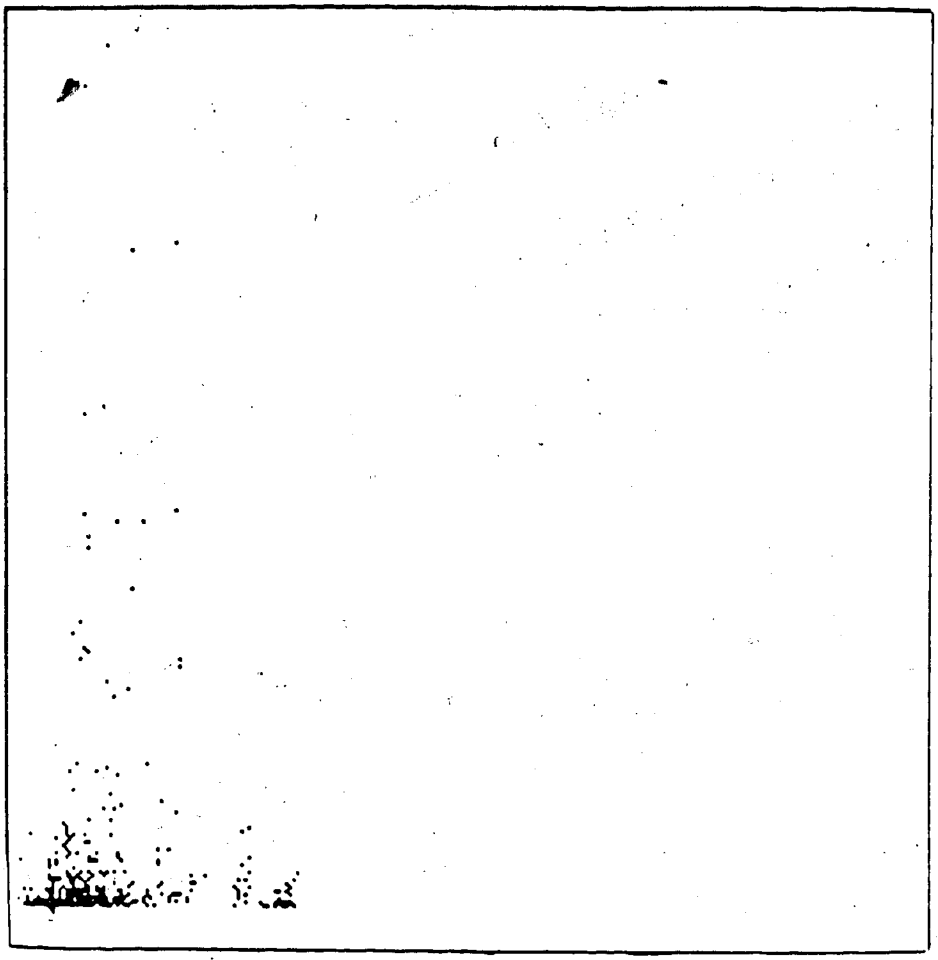

Figure 7-41 Swimming trial vocalization pattern recorded from Rat #5 (3% load)

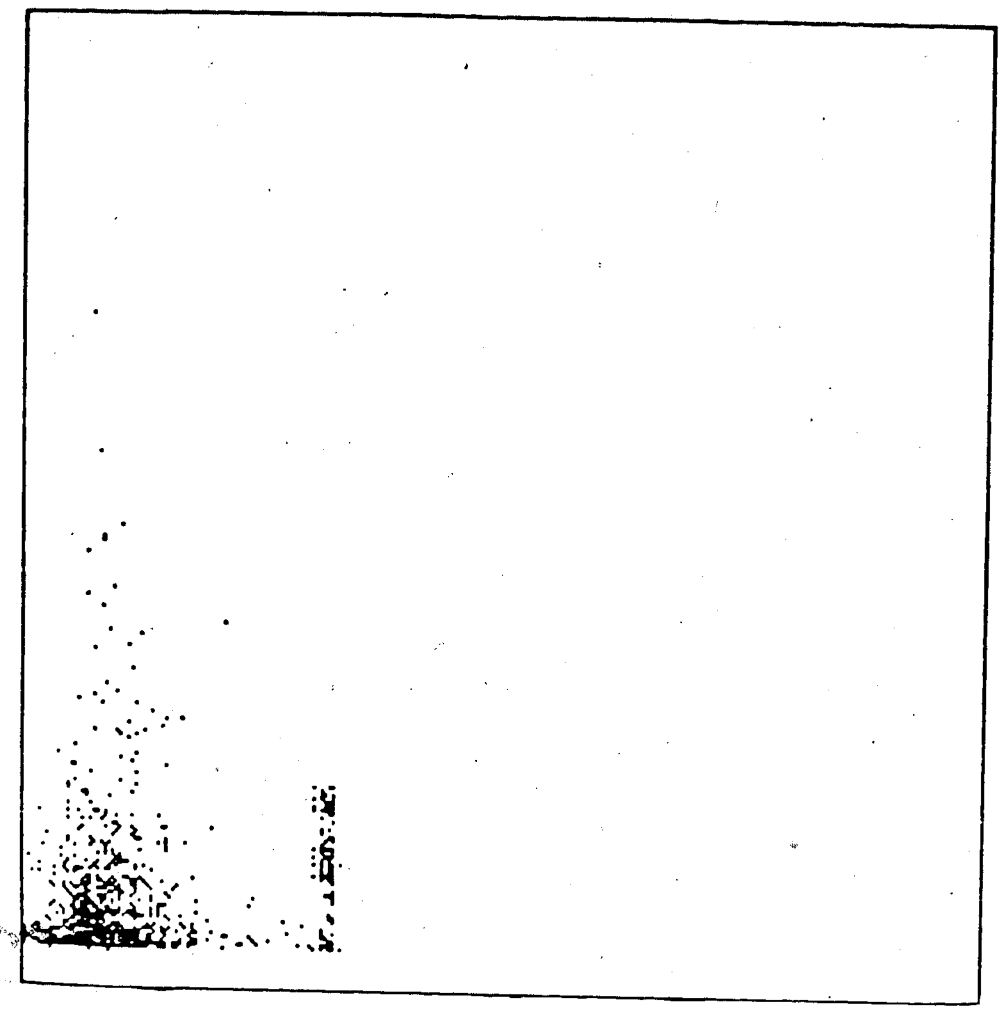

Figure 7-42 Swimming trial vocalization pattern recorded from Rats #7 and #8

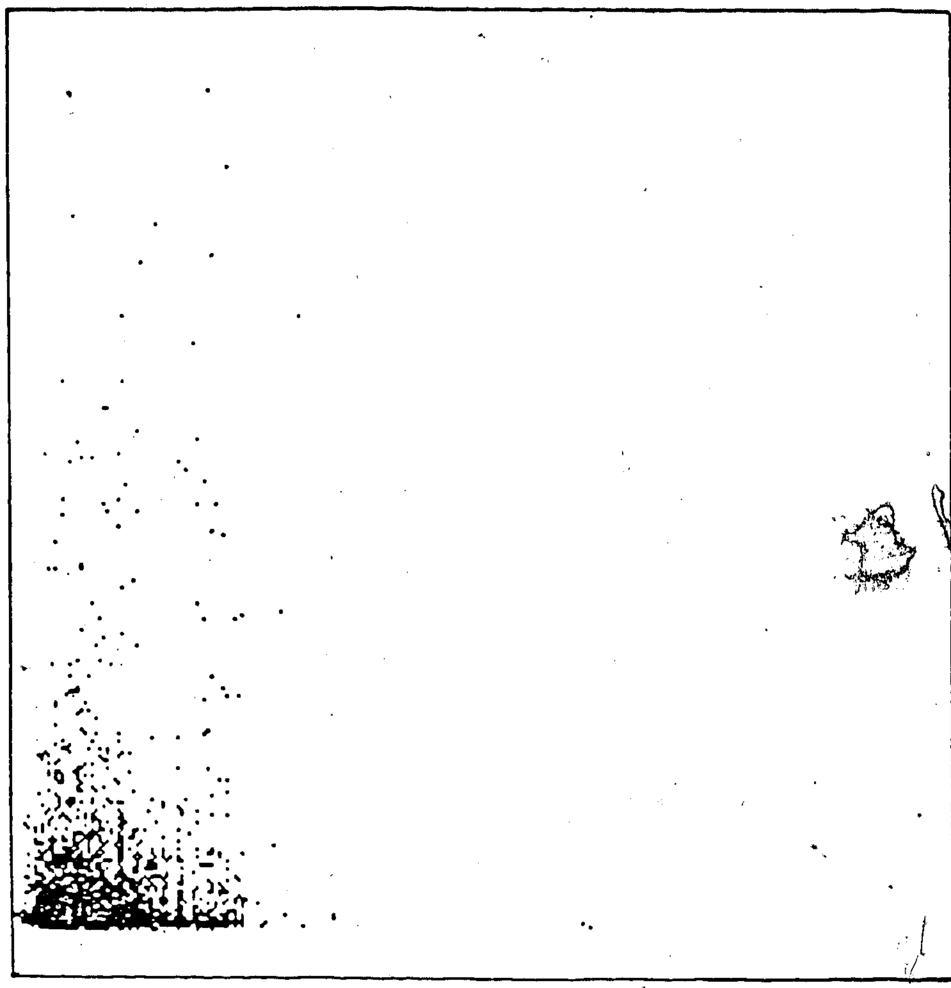

Figure 7-43 Swimming trial vocalization pattern recorded from Rats # 10 and # 11

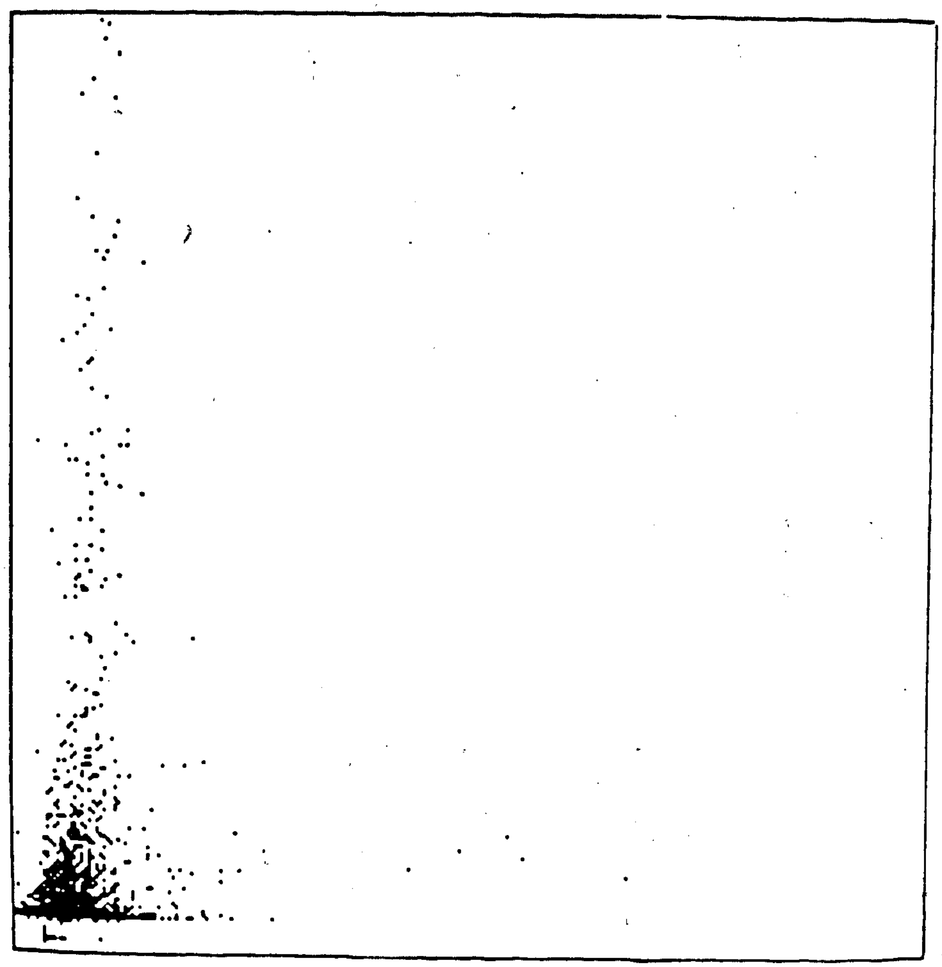

Figure 7-44 Swimming trial vocalization pattern recorded from Rat #11 (turbulent water conditions)

3. Other Trials

Even though the most intense vocalizations were produced by the rats subjected to acute exercise stress, vocalizations were also recorded under conditions in which no physical exercise stressor was present.

a. Vocalizations Recorded in Response to Stressed Animals

Stress calls were recorded from a large group of non-stressed rats when stressed rats (#2, #4) were alternatively placed in their immediate environment. These calls were virtually identical to the calls produced by the stressed rats. The calls occured spontaneously throughout the initial time intervals of the recording session. Thus it is evident that the vocalizations play an important role in communication between the rats.

b. Vocalizations Recorded From "Partner" Rats

At the completion of the treadmill training schedule, the rats were run for an additional session to facilitate the recording of vocalizations from the companion rats. The most intense stress vocalizations were recorded from rat #2 when rat #1 was running and from rats #4 and #5 when rat #3 was running. Since rats #2 and #4 performed poorly during the course of their training regimes, these vocalizations may be indicant of the rats' general predisposition to stress.

c. Vocalizations Recorded in Response to Treadmill Noise

At the completion of the treadmill training schedule, each participant rat was tested for vocalizations in response to noise generated by the treadmill. Two of the rats (#2, #4) emitted stress calls to a limited degree. These are the same two rats that performed poorly throughout the training sessions.

d. Vocalizations in Response to Artificially Reproduced Stress Calls

Two sessions were conducted in which rats were isolated from their respective cages. The first session involved rats #6, #7, and #8, with rat #8 being isolated; the second session involved rats #9, #10, and #11, with rat #11 being isolated. Psuedo stress calls were then artificially generated at 24 kHz. and the remaining rats in the cage were monitered for vocalizations. Stress calls were produced by only the male rats (#6, #7), however all of the rats exhibited an orientating response in which the rats would stand up against the side of the cage from which the vocalizations were originating.

D. Discussion

It is well documented that individuals cope with life events in a myriad of different ways. Friedman and Rosenman [1974] have attempted to correlate different copying styles with the incidence of heart disease. They postulate that individuals can be classified into two distinct groups, type A and type B, on the basis of their reactions to different stressors. Type A behavior is known as coronary prone behavior and is characterized by "an action-emotion complex that can be observed in any person who is aggresively involved in a chronic, incessant struggle to achieve more and more in less time, and if required to do so, against the opposing efforts of other things or persons"[Friedman&Rosenman, 1974]. Type B (noncoronary prone) behavior is identified by the lack of type A behavior. However, is this classification restricted only to members of our own species? The two distinct vocalization patterns recorded during the running trials exemplify the individuality with which rats react to a stressful situation. Thus, it may not be farfetched to assign the type A and type B labels to the two vocalization patterns. Rats vocalizing according to pattern II could be classified as type A. These rats consistently emitted high levels of stress vocalizations during the running trials. Rats with pattern I vocalizations could be classified as type B in that they were not adversely affected by the forced running situation.

Throughout the body of the thesis, it has been successively stated that the high frequency vocalizations can be used to assess the stress responsiveness of the rats. However, the exact frequencies at which the rats were vocalizing has not been discussed. It turns out that each rat vocalized at a particular frequency that remained fairly constant over the course of its training schedule. The frequencies at which each rat vocalized is given in table 7-6.

Table 7–6 Recorded Vocalization Frequencies

Rat #	Mean Vocalization Frequency	Comments
1	25.4	relatively few stress vocalizations
2	22.3	intense stress vocalizations
3	23.4	incidence of stress vocalizations increased towards the latter sessions
4	24.4	very intense vocalizations, refused to run on several occasions
5	22.3	sporadic vocalizations recorded during swimming trial
6	25.3	intense vocalizations recorded during the first session
7	26.4	running trials – intense vocalizations recorded towards the end of the recording session
7	34.1	swimming trial – distinct stress vocalizations in the middle of the session
8	34.1	" "
9	27.2	very few vocalizations scattered between 21 – 35 kHz.
10	–	no stress vocalizations were recorded for the running trial
10	22.3	sporadic stress calls were recorded during the swimming trial
11	22.3	" "

The 34 kHz. vocalizations produced by rats #7 and #8 are unique in that they are much higher pitched than the standard stress vocalizations found between 21 – 28 kHz. It may be that these higher pitched vocalizations are indicant of a distinct swimming distress call. However, why would forced swimming elicit a distinctly different stress call from that of forced running?

Vocalizations recorded from rat #7 during the running trials fell within the standard frequency bandwidth for stress calls. A comprehensive program involving the recording of vocalizations under a number of different situations may be able to verify whether or not different stressors elicit independant stress vocalizations.

VIII. Conclusion

The thesis could be considered as centering on two distinctly different themes:

1) an introduction to standard cluster analysis techniques and the development of the CLUSTREE routine as a viable alternative to these techniques,

2) the analysis of ultrasonic vocalizations as a measure of stress levels experienced by rats.

These two topics were linked by employing the CLUSTREE routine in the analysis of the recorded vocalization parameters.

The CLUSTREE technique was created due to the shortcomings of the standard clustering techniques. In comparison tests, it proved to be both an efficient and reliable counterpart to the standard techniques. The CLUSTREE technique was employed throughout all of the analysis phases and on the basis of the results it generated, it can be stated that the technique is very applicable to biological measurements. However, the applicablity of the CLUSTREE routine is not limited to strictly data analysis functions. It can also be employed in the behavioral sciences as a tool for modeling thought processes. This is accomplished by first simulating the thought process in terms of a stream of objects. These objects are then inputted in such a manner that the tree is updated after every k'th object enters the tree. The tree structure obtained at each step is representative of the changes elicited by the memory process.

One of the strongest points stated in this thesis was that stress levels experienced by rats could be quantified in terms of their high frequency vocalizations. This is supported by many researchers who have recorded ultrasonic vocalizations in response to many different stress situations. However, how is one certain that the vocalizations are actually stress calls rather than calls of some entirely different nature? In order to validate this, both physiological and vocalization measurements should be made in such a manner that they parallel each other. If the physiological data correlate well with the vocalization data, then there is a basis for ultrasonic vocalizations to be used as a assessment of stress levels. Such a basis would have many repercussions in the field of exercise physiology. Recording stress levels in terms of vocalizations would be a noninvasive alternative to techniques presently employed. In addition, the intensity of the vocalizations could be closely monitored such that if they exceed a certain level, the stressor eliciting these vocalizations is removed. This would permit research to be carried out without inflicting unnecessary cruelty on the animal, which is a primary concern in all realms of research that involve animals.

During the initial data analysis phase, it became evident that the stress vocalizations emitted by the majority of the rats were contained within a narrow frequency bandwidth between 21 and 28 kHz. This corresponds well with frequencies recorded by many other researchers. However, in some instances, vocalizations as high as 40 kHz. were recorded. Since the rats that emitted these higher frequency vocalizations were the same rats that experienced much difficulty with the training regime, it may be possible that these vocalizations are indicant of the rats' inability to effectively cope with the stressor. Hence these calls may be signifying that the rodent is, in a sense, giving up. If this is the case, then the experimenter should use these vocalizations as a signal to remove the stressor. However, due to the relatively small number of rats employed in the study, it is difficult to quantify the statistical validity of the higher frequency vocalizations being associated with only those rats experiencing much difficulty with the training regime.

One of the most significant findings was the distinct difference between the male and female stress vocalization patterns. Many researchers restrict themselves to using only male rats in the belief that the females' estrous cycles may influence their results. However, on the basis of the recorded stress vocalizations, it is highly indicative that the stress levels experienced by the males are more likely to contaminate results than the females' estrous cycles. The males emitted consistently higher quantities of stress calls then their female counterparts. Again, it is difficult to quantify the statistical validity of this finding due to the relatively small number of female rats employed in the study.

Another interesting finding was the apparent discrepancy between the vocalizations elicited in response to running and those elicited in response to swimming. On the basis of the number of stress vocalizations recorded during both the running and swimming trials, it appears that running is much more physically demanding than swimming. In fact, very few stress calls were recorded throughout the duration of the swimming trials. However, it seems logical that a significant number of stress vocalizations would be emitted at some point of the swimming session. Thus, if the durations of the sessions were increased until these stress vocalizations were recorded, the time difference between the onset of stress vocalizations for the running and swimming sessions could be employed as a measure of the relative intensities of the two training regimes.

It should be noted that because of space limitations, all of the trials took place in rooms containing many rats Thus, there is a possibility that the rats not directly exposed to any stressor may have been [illegible]ed by the stressed rats and vice versa. For this reason, any future trials should be conducted in such a manner that these possible interaction effects are eliminated.

The research conducted for this thesis has illuminated the need for further research to be carried out. Many possible research routes exist. Some of the research should be directed towards quantifying both the gender differences and the training method differences stated in the thesis. Research could also be directed towards examining the difference between vocalizations elicted from both trained and untrained rats. In addition, the analysis of ultrasonic vocalizations could provide a means of evaluating different training regimes.

However, before any new work is carried out, serious consideration should be given towards redesigning the digital ultrasound recorder. Although the recorder has more than surpassed its original design goals, problems were encountered during the data aquisition sessions. In many cases, the recorder's storage buffer became saturated while the recording session was in progress. Under such circumstances, data could no longer be recorded and thus the digital recording system must be reinitialized. This results in the loss of data between the time the buffer became saturated and the time the recorder is once again ***on line***. The recording system employed in future studies should be designed in such a manner that this problem can not possibly arise. Changes could also be made to the method in which new calls are labeled. The current method requires four numbers to label new calls. By reducing the label to only one number, an increase in data transmission efficiency is realized.

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Appendix 1

Over the past several decades, a number of stress models have been formulated. These stress models attempt to conceptualize the process(es) underlying the elicitation of the stress response. Most of the stress models differ significantly from one another. This may be partly due to the different treatments of the definition of the term stress. Since stress can encompass a wide variety of meanings, researchers tend to concentrate their efforts towards specific areas of stress research, and hence their models usually only apply to these specific areas.

The following models represent the directions that many researchers are following in their attempts to find the definitive stress model, *if such one exists.*

Model 1. One of the first stress models was formulated by the studies of Alexander[1950], Dunbar[1947] and Grinker and Spiegel[1945]. It is called the *psychosomatic model of stress* and is based on the premise that the tensions that occur in one system of the body often have detrimental consequences for other bodily systems. The psychosomatic model states that physiological reactions to stressful stimuli occur when the organism's responses are inappropriate. Also, the model states that there are two possible methods in which a solvable conflict can be handled. It can be handled directly in which case the tension produced by the conflict will be externally dissipated and systemic damage will most likely not occur. Otherwise, if the tension can not be directly handled, it will be internally dissipated in which case the likelihood of systemic damage is greatly increased. Anxiety or fear generated by serious conflicts can lead to either feelings of intense dread and discomfort or alterations in basic physiologic processes. The psychosomatic model is characterized by a chain reaction of the following consecutive states: the altered feeling state, the bodily adjustment state and the organic abnormality state (this state only occurs in situations in which the organism's responses are inadequate to cope with the stressor).

Model 2. Wolff[1953] formulated a stress model closely related to the psychosomatic model. It is based around the concept of a ***protective reaction pattern*** . The model states that the body reacts to physical stressors with a complex reaction (protective reaction pattern) aimed at first isolating the area of immediate threat, and then eliminating it completely. Unlike the psychosomatic model, Wolff's model states that altered feelings, bodily adjustment and behaviour occur simultaneously.

Model 3. One of the best known models is the *General Adaptation Syndrome* formulated by Selye[1950]. As previously discussed, Selye's model is characterized by its nonspecificity in that all stressors are always met with the same general reaction pattern. It can be called a biochemical model in that it is concerned with the analysis of stress at the physiological and biochemical levels of human function.

Model 4. Janis[1954] has formulated a model of stress that deals with the psychological responses of individuals to traumatic events. The model contains three basic elements:

- a disaster situation
- the psychological responses of individuals to disaster
- the situational determinants of these psychological responses.

Janis also goes on to identify three major phases of danger found in all large scale disaster situations. The first is the *threat phase* in which individuals realize that danger is imminent but the immediate environment is still free of this danger. The next phase is the *danger impact phase* during which individuals are confronted with the physical dangers. Lastly, in the *danger of victimization phase*, individuals assess the the losses that they have sustained. Four types of reactions are associated with these danger phases:

- apprehensive avoidance in which the individuals deny the existence of the situation

- stunned immobility which involves the loss of motor and mental activity
- docile dependancy in which individuals lack the ability to act independantly
- aggressive irritability in which individuals verbally attack anyone that irritates them

Even though the reactions usually produce different effects, they all result in a delpetion of mental activity.,

Two basic factors determine which type of reaction will occur, namely the perceived characteristics of the danger stimuli and the situational and predispositional determinants.

Model 5. Basowitz et al.[1955] have developed a model in which the relationships between stress and anxiety are examined. The model defines anxiety as a conscious and reportable experience of intense dread. These feelings are thought to arise when the soundness of the organism is threatened. Stress refers to the class of stimuli that are more likely to cause anxiety. The model ranks all stressful stimuli on a continuum based on the anxiety producing potential that the stimuli possess. The stimuli are grouped according to their potential for threat, not according to the variety of responses that they could provoke.

Model 6. Dohrenwend's[1956] model of stress involves the modification of Selye's model and is applied to the studies of mental disorders in the social environment. Dohrenwend bases his model around five basic sets of factors that he believes are involved in stressful situations:

- external stressors that throw the organism into an imbalanced state
- factors that mediate or relieve the effects of the stressor
- the experience of stress itself, which arises due to interactions between the stressor and mediating factors
- the adaptive syndrome which consists of the organism's attempt to cope with the stressor

- the organism's response which may either be adaptive or maladaptive

The model places much emphasis on the role of *mediating factors* in the stress response. Dohrenwend discriminates between two types of mediating factors, those that determine the amount of external constraint and those that determine the amount of internal constraint asociated with stress. These factors in turn produce conditions of external and internal control. External control occurs when force is directed towards activity of external events; internal control occurs when activity demanded by the external events is inhibited in favour of activity demanded by internal events. The model regards stress as *any* behaviour generated in response to pressures.

Model 7. Mechanic[1962] has formulated a model of stress around the social psychology of adaptation. In his model, Mechanic defines stress as the discomforting responses experienced by individuals in particular situations. Four factors determine whether or not a situation produces a discomforting response:

- the ability of the individual
- the skills and limitations created by group practices
- the environmental resources available to the individual
- the norms that define any conditions associated with the use of the resources

The term *reversibilty* is used to define the successful mastery of a situation and the feelings that are aroused in the process. Mechanic's model of stress applies to the social and social-psychological levels of functioning in the organism.

Model 8. The work by Mason[1971] does not actually formulate a new model of stress, but instead re-evaluates the concept of non-specificity and looks at new directions that stress research should follow. Mason believes that we should consider discrete stimuli and look at the specific effects that they produce. In doing so, Mason diminishes the need for stress concepts and terminology, particularly the non-specificity concept. He emphasizes the need to depart from the physiological oriented stress models in favour of a cognitive information processing design. Mason points out that " the primary mediator underlying the pituitary-adrenal cortical response to the diverse 'stressors' of early 'stress' research may simply be the psychological apparatus involved in emotional or arousal reactons to threatening or unpleasant factors in the life situation as a whole." On reviewing previous literature, Mason states that the roles of psychological processes in the stress response were usually not considered. "There seems to be little doubt that the potency of psychological influences in the regulation of corticosteroid levels was almost universally underestimated by early workers in the 'stress' field." Mason's efforts were directed towards establishing stress as a psychological and behavioral concept, not just a physiological one as many other researchers had previously labeled it.

Model 9. Lazarus[1971] has developed a stress model in which he emphasizes the psychological mechanisms underlying stress reactions. In this model he states that the anticipation of threat may elicit the same effects as the threat itself. Lazarus considers stress to be a general term which encompasses the environmental agents which disturb structure and function as well as responses to such agents. Thus a fundamental concept of stress is the relationship between the organism and the enviironment. "To speak as something as falling within the rubric of stress presumes a damaging transaction between some specific type of organism and some particular condition of the environment" [p 54]. Lazarus states that under conditions of harm or danger, the body mobilizes in attempt to *cope* with these conditions. Two types of coping processes are identified:

- *physiological* in which the neural and endocrine systems respond
- *psychological* in which the individual either pays selective attention to the non-threatening aspects of the environment or denies that danger even exists.

Lazarus acknowledges the existence of three realms of stress: social, psychological and physiological. Accordingly, stress can be defined as *any demands that tax the system (whether they be social,psychological or physiological) and the response of that system.* The model states that psychological stress depends upon the cognitive appraisal of a harmful, threatening or challenging event. Thus, in order to understand the factors underlying psychological stress, a full understanding of the cognitive processes mediating the stress response is required. In the case of physiological stress however, the condition of the tissues directly determines the presence of stress and thus the cognitive appraisal processes may be bypassed.

Lazarus has directed some of his efforts towards establishing the relationships between stress and disease. On a basic level, disease can be defined as some form of tissue damage or disturbance of function. However, it is also recognized that disease could refer to behavior disturbances in which no relative tissue damage occurs. Individuals are constantly coping to adapt to their environvent. If the coping processes are insufficient, then maladaptation can occur. Thus, disease can be thought of as a product of this maladaptation.

Lazarus[1974] subsequently extended his model in stating that " ... every instance of adaptive commerce between the person and the environment is appraised cognitively as to its significance for the person's well being. Such appraisals underlie the ebb and flow of *emotional states*, their quality and intensity" (italics added). Lazarus emphasises the major role that emotion plays in illness. Emotion arises from adaptive transactions that an individual has with his environment. Lazarus linked emotion with the cognitive information processing scheme in the stress response." The cornerstone of this psychological analysis of emotions is thus the concept of cognitive appraisal which expresses the evaluation of the significance of a transaction for the person's well being and the potentials for mastery in the continuous and constantly changing interplay between the person and the environmental stimulus configuration." Lazarus attends that the disease process may involve repeated emotional disturbances over a substantial period of time.

Model 10. A model developed mainly by Holmes and Rahe [1967] scaled various life events on the basis of their threat value. In their study of stress, Holmes and Rahe administered 'tests' in which individuals were to rank life events in terms of the threat value they elicited. Holmes and Rahe stated that individuals receiving high scores on the 'tests' were more likely to become ill than those individuals receiving low scores. Subsequent research (Holmes & Masuda, 1973) attempted to establish the level of correlation between 'test' results and disease.

In the process of their research, they noted that one common element was usually associated with each life event. This element was the deployment of adaptive or coping mechanisms in response to the life events. Thus, Holmes and Rahe viewed stress as anything that elicited the coping mechanisms.

Model 11. Dohrenwend and Dohrenwend[1972] have formulated a psychosocial model of stress which makes a distinction between a stressor and the state of stress which is the immediate reaction to the stressor (figure 11). However,they mention that this state of stress is usually inferred rather than observed. The model suggests that the events that follow the state of stress depend upon the mediation of situational (external) and personal (internal) factors. These factors give rise to three possible outcomes:

- psychological growth
- no substantial change in functioning
- adverse change in functioning

The model carefully points out that the occurence of -ss does not necesarily imply the existence of stress. Changes in health and behavior can arise from other causes." For example, if a person who is a poor member of a disadvantaged ethnic group develops pneumonia, it does not follow that psychosocial stress must have been involved. Poor nutrition, heavy alcohol consumption, and a hard winter in unheated housing might well be sufficient. In general, in the absence of evidence that a person has already experienced one or more stressful life events, one cannot infer that his dysfunctional behavior or illness was induced by psychosocial stress"

Model 12. A recent model by Burchfield[1979] attends that all organisms are genetically predisposed to accomodate stress. In the model, Burchfield states that the perception of an event is necessary before it can be classified as a stressor. Once the event has been perceived, it may either be consciously or unconsciously attended to. Purely physical stimuli are not regarded as stressors in the definition formulated above. They may require physiological adaptation, however psychological functioning is not affected. Burchfield observes that psychological changes are usually accompanyed by alterations in physiological homeostatic responses.

Burchfield differentiates between three different types of stressors:

- *Acute Stress* which can be defined as a short term event which does not frequently reoccur
- *Chronic Intermittent Stress* which can be defined as repeat exposure to a discrete stimulus for a specified amount of time
- *Chronic Stress* in which the organism is continuously exposed to a stimulus

Burchfield concerns herself with chronic intermittent stress and the responses that it elicits. Before formulating an explicit model of stress, Burchfield summarizes the stress response as follows:

- the initial confrontation between the organism and a stressor elicits sympathetic arousal and adrenocortical secretion
- repeated exposure decreases the sympathetic arousal
- repeated exposure creates an orienting, anticipatory response
- after repeated exposure to one stressor, a new stressor will elicit the same response as the original occurence of the first stressor

- after repeated exposure, the orienting response will start the response before the onset of the stressor in such a manner that maximum response occurs prior to stressor exposure, instead of during it.

From the above framework, it is possible to formulate a model for adaptation to chronic intermittent stress. The model consists of the successful fulfillment of three goals:

1) maintenance of homeostasis
2) conservation of resources
3) effective defense against stress.

If all of these conditions are satisfied, then the organism is said to be *maximally fit*. Under conditions of acute stress, the organism can quite readily achieve all three goals with a minimum usage of resources. However, the same response to conditions of chronic intermittent stress would eventually result in the depletion of available resources. In such a case, homeostasis would be lost. A more effective response would involve a minimal usage of resources while still maintaining adequate defense. In order to facilitate such a response, the organism must learn to anticipate the onset of stress and act accordingly. By initiating the response prior to the occurance of any stressful stimuli, resources can be mobilized beforehand and the stressor may be effectively controlled.

Model 13. Weisfeld[1982] formulated a model by looking at the existing models and identifying the areas in which they are inadequate. Weisfeld refers to the General Adaptation Syndrome as an explanation of the stress response on a physiological or *proximate causation* level. He states that the current concept in which psychological factors mediate physiological responses to a stressor can also be be veiwed as an expalanation on the same level. Weisfeld states that there is another level of explanation, namely *ultimate causation* which refers to evolutionary function. This level suggests that physiological systems and behavioral patterns are adaptive in that they facilitate the organism's survival and reproduction.

Weisfeld disagrees with the G–A–S in that he views death as occuring despite the organsim's compensatory processes. In the stage of exhaustion, Selye[1950] views death as occuring due to these compensatory processes. Weisfeld states that the G–A–S should be appreciated only in terms of its adaptive processes.

Weisfeld also disagrees with Burchfield's[1979] model. He argues that even if an organism achieves all the goals required to be being maximally fit, it will not necessarily be *biologically fit*. "No matter how effectively an organism accomplished these metabolic goals, however, its biological fitness would be zero unless it succeeded in performing the behaviors of feeding and reproduction. These behaviors do not neatly reduce to the metabolic level; they must be recognized as essential on the organismic level" [p 82]. Thus Weisfeld states that the behavioral and physiological levels must be integrated before an accurate model of stress can be formulated.

Weisfeld's model is based on the contention that all organisms must perform four functions during their life span:

1) defense
2) feeding
3) reproduction
4) rest

Each of the functions is essential for the existence of the organism.

The model views reactions to stressors as a temporary shift in biological priorities to the defense function. The model is not considered to depict a homeostatic system, but rather a *polystatic* system in which there is no normal testing state.

Appendix 2

Program CLUSTREE for Three-Distance Clustering

```
program clustree;

(   created         -  Sep  12, 1984
    last revision      July 22, 1985

    purpose  -  an efficient counterpart to
                standard clustering methods

    input    -  # of objects to be clustered; dimension
                of the measurement space, the objects
                to be clustered
                (unit=scards)

             -  interactive data for which the user
                is prompted (unit=terminal)

    output   -  clustering iterations, final tree
                structure,
                (unit=sprint)
                                                        )

label 1,2,3                                          ;

type t1=packed array[1..10] of real                  ;
     a1=array[1..300] of real                        ;
     a2=array[1..2000] of integer                    ;
     a3=array[1..2000] of t1                         ;
     a4=packed array[1..2000] of integer             ;
     a5=packed array[0..128] of char                 ;
     a6=       array[0..128] of a5                   ;
     a7=packed array[0..20] of char                  ;
     a8=array[1..50] of integer                      ;
     a9=array[1..14,1..2000] of char                 ;

     c1=(move,leaf,break,dump)                       ;
     z1=set of 1..255                                ;

     ptr  = ^node                                    ;
     node = record
              num,level              : integer       ;
              dl,dr,d3               : real          ;
              newvert                : t1            ;
              elements               : a4            ;
              left,right,prev        : ptr           ;
            end; (record)

var  i,j,k,kn,l,nl,nm,ll,rl,nitt
     ,m,nobject,dim,elem,maxnitt
     ,total,l2,r2,corr,incorr,xm
     ,ym,xl,yl,fx,fy,preset,nlev
     ,ndo,vi,length,max,dummy
     ,metric                          : integer      ;
     data,temp,templ,temp2
     ,templ,tempr                     : t1           ;
     vincorr,
     vcorr,next,tvert,tv1,
     pathlen,tempor                   : a2           ;
     datum                            : a3           ;
     grey                             : a6           ;
     str1,str2                        : a7           ;
     lev,la                           : a8           ;
     larr                             : a9           ;
     graphreq,
     d3req,update,cont,clusmem        : boolean      ;
     dec                              : c1           ;
     dis,dmin,percent                 : real         ;
     v1,v2,v3,v4,cm,gr,ds             : char         ;
     root,p1,p,q,r,s                  : ptr          ;
     term_in,term_out                 : text         ;

procedure title;

var i : integer;

begin
  for i:=1 to 4 do
      writeln;
  writeln('CCCCCCC':8,'LL':4,'UU':9,'UU':6,'SSSSSSS':9,
          '  TTTTTTTT RRRRRRRR  EEEEEEEE EEEEEEEE');
  writeln('CCCCCCCCC','LL':3,'UU':9,'UU':6,' SSSSSSSSS',
          'TTTTTTTT RRRRRRRRR EEEEEEEE EEEEEEEE');
  writeln('CC','CC LL':10,'UU':9,'UU SS':9,'SS':7,'TT':6,
          'RR':6,'RR EE':10,'EE':9);
  writeln('CC','LL':10,'UU':9,'UU SS':9,'TT':13,'RR':6,
          'RR EE':10,'EE':9);
  writeln('CC','LL':10,'UU':9,'UU SSSSSSSS':15,'TT':7,
          'RRRRRRRRR':1J,' EEEEEEEE EEEEEEEE');
  writeln('CC','LL':10,'UU':9,'UU  SSSSSSSS':16,'TT':6,
          'RRRRRRRR':12,'  EEEEEEEE EEEEEEEE');
  writeln('CC','LL':10,'UU':9,'UU':6,'SS':10,'TT':6,'RR':6,
          'RR':5,'EE':5,'EE':9);
  writeln('CC','CC LL':10,'LL UU':9,'UU SS':9,'SS':7,'TT':6,
          'RR':6,'RR':5,'EE':5,'EE':9);
  writeln('CCCCCCCCC LLLLLLLL UUUUUUUU SSSSSSSSS','TT':6,
          'RR':6,'RR ':7,' EEEEEEEE EEEEEEEE');
  writeln(' CCCCCCC  LLLLLLLL UUUUUUUU  SSSSSSS','TT':7,
          'RR':6,'RR ':7,' EEEEEEEE EEEEEEEE');
  for i:=1 to 2 do
      writeln;
  writeln('H I E R A R C H I C A L    C L U S T E R I N G',
          '      P A C K A G E');
  for i:=1 to 4 do
      writeln;
  writeln('RELEASE  1.0  FOR  MTS');
  writeln('July, 1985');
  for i:=1 to 2 do
      writeln;
  writeln('DEVELOPED BY MICHAEL SLAWNYCH');
  writeln('DEPARTMENT OF ELECTRICAL ENGINEERING');
  writeln('THE UNIVERSITY OF ALBERTA');
end;

{                                                   }
{                                                   }
{                                                   }
{                                                          }
{                                                          }
{                                                          }
procedure func(var p:ptr; i,l, elem,dim integer,
                                var temp t1);
```

```
var  j   : integer        ;
begin
     for j:=1 to dim do
        temp[j]:=(p^.newvert[j]*p^.num+n[j]*elem)/
                                        (p^.num+elem)
end;
{                                                  }
{                                                  }
{                                                  }
procedure dist(m,n:t1; i,dim:intege  var dis real);
var   j,k,l            integer;
      s1,s2,s3,s4   real    ;
begin
    dis:=0;
    s1:=0;
    s2:=0;
    s3:=0;
    case i of
  1:  begin
        for j:=1 to dim do
             dis:=dis+abs(m[j]-n[j]);
      end;
  2:  begin
        for j:=1 to dim do
             dis:=dis+(m[j]-n[j])*(m[j]-n[j]);
        dis:=sqrt(dis);
      end;
  3:  begin
        for j:=1 to dim do
             begin
                s4:=1;
                for k:=1 to i do
                     s4:=s4*(m[j]-n[j]);
                dis:=dis+s4;
             end;
      end;
  4:  begin
        for j:=1 to dim do
             if abs(m[j]-n[j])>dis
                then dis:=abs(m[j]-n[j]);
      end;
  5:  begin
        for j:=1 to dim do
             begin
                s1:=s1+m[j]*n[j];
                s2:=s2+m[j]*m[j];
                s3:=s3+n[j]*n[j];
             end;
        dis:=s1/s2/s3;
      end;
   end; {case}
end;
{                                                  }
{                                                  }
{                                                  }

function scan(l,m integer; var arr a4) boolean;

var k         integer;
    ok        boolean;
begin
   ok:=false;
   k:=1;
   while (not ok) and (k<=m) do
          if arr[k]=1
             then ok:=true
             else k:=k+1;
   scan:=ok;
end;


procedure remove(l,m:integer;var arr a4);

var j,k      : integer;
    loc      : boolean;

begin
   loc:=false;
   k:=1;
   while not loc do
       if arr[k] = 1
          then loc:=true
          else k:=k+1;
   for j:=(k+1) to m do
       arr[j-1]:=arr[j];
end;
procedure deciss(var p:ptr; dim,max integer; var dec ct;
                          d3req,update boolean; temp:t1);

var i,j                                   integer;
    l1,r1,q                               ptr    ;
    temp1,temp2                           t1     ;
    dis1,dis2,dis3,dtemp,dmin             real   ;
begin
   l1:= p^.left;
   r1:= p^.right;
   if p^.level = max
      then dec:=dump
      else if l1=nil
             then dec:=leaf
             else
               begin
                 q:=p;
                 dist(l1^.newvert,temp.metric,dim,p^.dl);
                 dist(r1^.newvert,temp.metric,dim,p^.dr);
                 dist(l1^.newvert,r1^.newvert,metric,dim,dis3).
          dtemp:=0;
          if d3req
             then if l1^.d3 > r1^.d3
                       then dtemp:=l1^.d3
                       else dtemp:=r1^.d3;
          dis3:=dis3+dtemp;
          dmin:=p^.dl;
          if p^.dr < dmin
             then dmin:=p^.dr;
          if dis3 < dmin
             then dmin:=dis3;
          if p^.dl = dmin
             then
```

```
          begin
            p:=l1;
            dec:=move;
          end
        else if p^.dr = dmin
          then
            begin
              p:=r1;
              dec:=move;
            end
          else dec:=break;
      if d3req and update and (q^.d3<>dmin)
        then if p^.d3 <dmin
              then p^.d3 :=dmin;
    end;
end;
{                                              }
{                                              }
{                                              }
procedure search(var dim,max,nobject,corr,incorr :integer;
           var vcorr,vincorr:a2; d3req,update : boolean;
                                          var head:ptr);

label                                 1;
var    j,k,l                     :integer;
       dec                       :c1     ;
       temp,templ                :t1     ;
       p,q                       :ptr    ;
{                                              }
{                                              }
{                                              }
begin
    l:=0;
    corr:=0;
    incorr:=0;
    while l< nobject do
    begin
        l:=l+1;
        p:=head;
    1:  deciss(p,dim,max,dec,d3req,update,datum[l]);
        case dec of
 move:  goto 1;
 dump,leaf:  begin
            if scan(l,p^.num,p^.elements) then
              begin
                corr:=corr+1;
                vcorr[corr]:=l;
              end
            else
              begin
                incorr:=incorr+1;
                vincorr[incorr]:=l;
              end
        end;
 break  begin
            incorr:=incorr+1;
            vincorr[incorr]:=l;
        end;
      end  { case statement completed }
    end    { while loop completed     }
```

```
end;


procedure change(p:ptr ; n:integer);

begin
  if p <> nil
    then
      begin
        p^.level:=p^.level+n;
        change(p^.left,n);
        change(p^.right,n)
      end
end

procedure delete(var p1 :ptr; incorr,dim :integer;
                                    vincorr:a2);

var     l,m         : integer;
        p,q,s       : ptr    ;
        cont        : boolean;

begin
  for l:=1 to incorr do
      begin
        p:=p1;
        cont:=true;
        while cont do
          begin
            for m:=1 to dim do
                p^.newvert[m]:=(p^.newvert[m]*p^.num-
                               datum[ vincorr[l], m])/(p^.num-1);
            remove(vincorr[l],p^.num,p^.elements);
            p^.num:=p^.num-1;
            q:=p^.left;
            if scan(vincorr[l],q^.num,q^.elements)
              then p:=q
              else p:=p^.right;
            if p^.left = nil
              then if p^.num = 1
                then
                  begin
                    q:=p^.prev;
                    if q^.left = p
                      then s :=q^.right
                      else s :=q^.left;
                    r:=q^.prev;
                    if r^.left = q
                      then r^.left :=s
                      else r^.right :=s;
                    s^.prev :=r;
                    change(s, 1);
                    cont:=false;
                  end
                else
                  begin
                    func(p,datum[ vincorr[l] ],  l,dim
                                                 p^.newvert)
```

```
                    remove(vincorr[i],p^.num,p^.elements);
                    p^.num:=p^.num-1;
                    cont:=false;
                  end;
             end
         end
end;

procedure max_check(var p:ptr; max:integer);

begin
   if p <> nil
   then
     begin
       if p^.level = max
          then if p^.left <> nil
               then
                 begin
                   p^.left:=nil;
                   p^.right:=nil;
                 end;
       max_check(p^.left,max);
       max_check(p^.right,max);
     end
end;


procedure reset_grey(var grey:a6; xm,ym,xl,yl:integer);

var i,j    :integer;

begin
  for i:=0 to ym do
      for j:=0 to xm do
          grey[i,j]:=' ';
  for i:=0 to ym do
      begin
        grey[i,0]:='+';
        grey[i,xm]:='+'
      end;
  for i:=1 to (xm-1) do
      begin
        grey[0,i]:='+';
        grey[ym,i]:='+'
      end;
  for i:=1 to ym div yl do
      for j:=1 to (xm-1) do
          grey[yl*i,j]:=' ';
  for i:=1 to xm div xl do
      for j:=1 to (ym-1) do
          grey[j,xl*i]:=' ';
end;

procedure setup(p:ptr; var grey:a6; fx,fy:integer; var k:integer);

var i,j,k1,k2    :integer;
            a    :char   ;

begin
  case k of
     1: a:='1';
     2: a:='2';
     3: a:='3';
     4: a:='4';
     5: a:='5';
     6: a:='6';
     7: a:='7';
     8: a:='8';
     9: a:='9';
    10: a:='a';
    11: a:='b';
    12: a:='c';
    13: a:='d';
    14: a:='e';
    15: a:='+';
    16: a:='*';
     end; (case)
   for i:=1 to p^.num do
       begin
         j:=p^.elements[i];
         k1:=round(datum[j,1]);
         k2:=round(datum[j,2]);
         grey[ k1 div fy , k2 div fx ]:=a
       end;
end;
procedure outputclus(grey:a6; xm,ym,xl,yl,fx,fy:integer);

var i,j,k1;k2   : integer;

begin
  writeln('1');
  writeln;
  writeln('   x axis        F R E Q U E N C Y');
  writeln('   y axis        A M P L I T U D E');
  writeln;
  write('  ');
  for i:=0 to xm div xl do
      write(xl*i*fx:xl);
      writeln;
  for i:=0 to ym-1 do
      begin
        if i mod yl = 0
          then write(i*  :xl-1,'  ')
          else write('  ':xl);
        for j:=0 to xm do
            write(grey[i,j]);
        writeln;
      end;
  write(ym*fy:xl-1,'  ');
  for i:=0 to xm do
      write(grey[ym,i]);
  writeln;
end;


procedure set_table;

begin
  write('  ------------                 ');
```

```
    writeln('                                              ');
    writeln;
    writeln(' ':20,'    T R E E    S T R U C T U R E ');
    writeln;
    write('                                               ');
    writeln('                                             ');
    writeln;
    write('   vertex      vertex    ');
    writeln('   # of       vector');
    write('   level       type      ');
    writeln('   elms       components');
    writeln('
                                                   ');
end;

procedure display1(p:ptr; m,preset,j,fx,fy:integer;
                   var k:integer; graphreq:boolean);

var  i : integer;
begin
  if p <> nil
    then
      begin
        if (p^.level <= j) and (p^.level <> 0)
          then  writeln('
                                                 ');
        write(p^.level:7,' ':5);
        if p^.prev=nil
          then  write('   root')
            else if p^.left<>nil
              then write('    vert')
                else if p^.num=1
                  then write('    leaf')
                    else write('    dump');
        write(p^.num:10,' ':3);
        for i:=1 to m do
            write(p^.newvert[i]:8:3);
        writeln;
        if (p^.level = preset) and graphreq
          then
            begin
              k:=k+1;
              setup(p,grey,fx,fy,k);
            end;
        display1(p^.left,m,preset,j,fx,fy,k,graphreq);
        display1(p^.right,m,preset,j,fx,fy,k,graphreq);
      end
end;

procedure display2(p:ptr; preset:integer; var j:integer;
                                          var larr:a9);

var  i : integer;
begin
  if p <> nil
    then
      begin
        if p^.level = preset
          then
            begin
              for i :=1 to p^.num do
                begin
                  larr[j,p^.elements[i]] :='x';
                end;
              j:=j+1;
            end;
          display2(p^.left,preset,j,larr);
          display2(p^.right,preset,j,larr);
      end
end;
{                                                            }
{                                                            }
{                                                            }
begin { m a i n   p r o g r a m       }
{                                                            }
{                                                            }
{                                                            }
      reset(term_in,    'file=*msource* interactive');
      rewrite(term_out, 'file=*msink*');
      title;
      page;
      new(p);
      new(q);
      new(r);
      d3req:=true;
      update:=true;
      clusmem:=false;
      graphreq:=false;
      nitt:=0;
      for j:=1 to 14 do
          for i:=1 to 2000 do
              larr[j,i] :=' ';
      la[1]:=1;
      la[2]:=3;
      la[3]:=7;
      writeln(term_out,' input the follwing  ');
      writeln(term_out,'   max  level for tree,',
                  '    max  number of iterations,',
                  '    distance metric');
      readln(term_in,max,maxnitt,metric);
      readln(j,dim);
      for i:=1 to j do
        begin
        { read(dummy,dummy,dummy); }
          for l:=1 to dim do
              read(datum[i,l]);
          readln;
        end;
      nobject :=j;
      ndo:=j;
      for i:=1 to nobject do
          next[i]  := i;
      nl:=next[1];
      nm:=next[2];
      root :=p;
      root^.left :=q;
      root^.right :=r;
      root^.num :=2;
```

```
      root^.level:=0;
      root^.elements[1]:=1;
      root^.elements[2]:=2;
      root^.prev:=nil;
      q^.left:=nil;
      r^.left:=nil;
      q^.right:=nil;
      r^.right:=nil;
      q^.num:=1;
      r^.num:=1;
      q^.level:=1;
      r^.level:=1;
      q^.elements[1]:=1;
      r^.elements[1]:=2;
      q^.prev:=root;
      r^.prev:=root;
      q^.d3:=0;
      r^.d3:=0;
      for l:=1 to dim do
          root^.newvert[l]:=(datum[nl,l]+datum[nm,l])/2.
      q^.newvert:=datum[nl];
      r^.newvert:=datum[nm];
      if d3req then
          dist(datum[nl],datum[nm],metric,dim,root^.d3);
      l:=2;
   2: update:=true;
      while l <= (ndo-1) do
      begin
          l := l+1;
          nl:= next[l];
          p := root;
      1:  func(p,datum[nl],1,dim,temp);
          declss(p,dim,max,dec,d3req,update,datum[nl]);
          case dec of
move:     begin
              q:=p^.prev;
              q^.num:=q^.num+1;
              q^.elements[q^.num] :=nl;
              q^.newvert:=temp;
              goto 1
          end;
leaf:     begin
              new(r);
              new(s);
              q:=p^.prev;
              r^.prev:=q;
              r^.left:=p;
              if q^.left=p
                 then q^.left   := r
                 else q^.right  := r;
              r^.right:=s;
              r^.num:=p^.num+1;
              for k:=1 to p^.num do
                  r^.elements[k]:=p^.elements[k];
              r^.elements[r^.num]:=nl;
              r^.newvert:=temp;
              r^.level:=p^.level;
              p^.level:=p^.level+1;
              p^.prev:=r;
              s^.prev:=r;
              s^.left:=nil;
              s^.right:=nil;
              s^.num:=1;
              s^.elements[1]:=nl;
              s^.newvert:=datum[nl];
              s^.level:=p^.level;
              s^.dl:=0;
              s^.dr:=0;
              s^.d3:=0;
             (r^.d3:=0;)
              dist(p^.newvert,s^.newvert,metric,dim,r^.d3)
          end;
break:    begin
              new(r);
              new(s);
              if p=root
                 then
                   begin
                     root:=r;
                     r^.left:=p;
                     r^.right:=s;
                     r^.prev:=nil;
                     r^.level:=0;
                   end
                 else
                   begin
                     q:=p^.prev;
                     r^.left:=p;
                     if q^.left=p
                        then q^.left   := r
                        else q^.right  := r;
                     r^.right:=s;
                     r^.prev:=q;
                   end;
              r^.elements:=p^.elements;
              r^.num:=p^.num+1;
              r^.elements[r^.num]:=nl;
              r^.newvert:=temp;
              r^.level:=p^.level;
              p^.prev:=r;
              s^.prev:=r;
              s^.left:=nil;
              s^.right:=nil;
              s^.num:=1;
              s^.elements[1]:=nl;
              s^.newvert:=datum[nl];
              s^.level:=r^.level+1;
              s^.dl:=0;
              s^.dr:=0;
              s^.d3:=0;
              dist(p^.newvert,s^.newvert,metric,dim,r^.d3);
              change(p,1);
              p1:=root;
              max_check(p1,max);
          end;
dump      begin
            p^.newvert:=temp;
            p^.num:=p^.num+1;
            p^.elements[p^.num]:=nl;
          end;
```

```
   end  {case statement completed}
  end;

  update:=false;
  writeln;
  write('  * * * * * * * * * * * * *');
  write('   i t e r a t i o n   n o    ',nitt:3,'    3);
  writeln('* * * * * * * * * * * * * *');
  writeln;
  search(dim,max,nobject,corr,incorr,vcorr,vincorr,
                       d3req,update,root);
  write('  number of correct realizations    ',corr:3);
  writeln('         number of incorrect realizations  ',incorr:3);
  writeln;
  if incorr<>0 then
  begin
      if incorr>(nobject/2)
      then
         begin
            writeln('  error level is critical  ',
                    '--  process terminated ');
            goto 3
         end;
      delete(root,incorr,dim,vincorr);
      writeln;
      write('  the following element(s) could not be  ');
      writeln('located in the tree');
      vi:=2;
      for i:=1 to incorr do
      begin
        if (i-1) mod 25 = 0
           then writeln;
        write(vincorr[i]:4);
        next[i+1]:=vincorr[i];
      end;
      writeln;
      writeln;
      percent:=corr/nobject*100;
      writeln('  percentage of objects that found their
               position = %',percent:7:3);
      writeln;
      writeln;
  {   check(newvert,elm,vleft,vright,d3,dleft,dright,
                           nobject,dim,clust)  }
      ndo:=incorr+1;
      i:=1;
      nitt:=nitt+1;
      if nitt<=maxnitt
         then goto 2
   end;
  writeln;
  writeln;
  writeln(term_out,'is a cluster membership required?',
                  '(  (y)es or (n)o  )');
  readln(term_in,cm);
  if (cm='y') or (cm='Y')
    then
      begin
        clusmem:=true;

      writeln(term_out,'  input the number of levels
      ,        ' and the particular levels required ');
      read(term_in,nlev);
      for i:=1 to nlev do
          read(term_in,lev[i]);
      readln(term_in);
    end;
  xm:=128;
  ym:=128;
  xi:=8;
  yi:=4;
  fx:=1;
  fy:=2;
  preset:=1;
  writeln(term_out,' is a graph required?');
  readln(term_in,gr);
  if (gr='y') or (gr='Y')
     then
       begin
         graphreq:=true;
         writeln(term_out,'  default set up?');
         readln(term_in,ds);
         if (ds<>'y') and (ds<>'Y')
            then
              begin
                writeln(term_out,'input - x and y dimensions
                ' x and y scale markings, x and y div factors ');
                readln(term_in,xm,ym,xi,yi,fx,fy);
                writeln(term_out,'cluster level to be
                          ' graphed?');
                readln(term_in,preset);
          {  yi:=xi*round(fx/fy);  }
              end;
       end;
  if graphreq
     then reset_grey(grey,xm,ym,xi,yi);
  page;
  set_table;
  k:=0;
  display1(root,dim,preset,2,fx,fy,k,graphreq);
  page;
  if clusmem
     then
       begin
         writeln;
         write('                    ');
         write(' C L U S T E R    M E M B E R S H I P ');
         writeln('                    ');
         writeln;
         writeln('  O B J E C T ',' ':dim*5,' C L U S T E R ')
         writeln;
         writeln(' ':43+dim*5,' 1  1  1  1  1');
         writeln(' ':13+dim*5,' 1 2       3 4 5 6
                          7 8 9 0 1 2 3 4 ');
         writeln;
         for i:=1 to nobject do
            begin
              write(i:4,'  ');
              for j:=1 to dim do
                  write(datum[i,j]:5:1);
```

```
                for k:=1 to nlev do
                    begin
                      i:=la[k];
                      display2(root,lev[k],i,larr);
                    end;
                write(' ':6);
                for k:=1 to 2 do
                    write(larr[k,1]:2);
                write(' ':6);
                if nlev>=2
                   then for k:=3 to 6 do
                     write(larr[k,1]:2);
                write(' ':6);
                if nlev>=3
                   then for k:=7 to 14 do
                     write(larr[k,1]:2);
                writeln;
           end;
       end;
   if graphreq
      then outputclus(grey,xm,ym,xl,yl,fx,fy);
3:    ;
end
```

Appendix 3

Pascal and Fortran Routines

```
program readdata;

   created      Oct 10, 1984
  revised      JAn 15, 1985

  purpose -  to format the digital ultrasound
             recorder output in such a manner
             that data analysis packages  can
             be directly applied

  input   -  digital ultrasound recorder output
             (unit=scards)

          -  interactive data for which the user
             is prompted (unit=terminal)

  output  -  call no., call duration, call ampl
             & s_dev ampl., call freq. & s_dev
             freq

type  a2=array[1..40000] of integer;
      ptr  = ^node;
      cell = record
                head,tail : ptr    ;
                time,dur  : integer;
                ampl,freq,
                adev,fdev : real   ;
             end;(record)
      node = record
                d1,d2:integer;
                next:ptr;
             end;(record)
      r1=array[1..1200] of cell     ;
      e1=(similar,noise_check)      ;
      s1=packed array[1..30] of char;
      s2=packed array[1..20] of char;
var   data                          : a2    ;
      call                          : r1    ;
      criterion                     : e1    ;
      time_date,rat_info            : s1    ;
      term_in,term_out              : text  ;
      ease,n1,n2,
      min_level,run_dura,run_vel,
      ncall,i,j,k,l,m,n,count,newcall   : integer;

procedure addqueue(var call:r1; i,n1,n2:integer);

var p:ptr;
begin
   with call[i] do
      begin
         new(p);
         p^.d1:=n1;
         p^.d2:=n2;
         p^.next:=nil;
         if tail=nil
            then
               begin
                  tail:=p;
                  head:=p;
               end
            else
               begin
                  tail^.next:=p;
                  tail:=p;
               end
      end
end;

procedure view_q(p1:ptr);

var p:ptr;
begin
   p:=p1;
   while p<>nil do
      begin
         write(p^.d1:4,p^.d2:4);
         p:=p^.next
      end;
   writeln;
   writeln
end;

procedure stan_dev(p1:ptr; var call:r1; i:integer);

var p:ptr;
begin
  with call[i] do
     begin
        adev:=0.0;
        fdev:=0.0;
        if dur <> 1
           then
              begin
                 p:=p1;
                 while p<>nil do
                    begin
                       adev:=adev+(p^.d1-ampl)*
                              (p^.d1-ampl)/(dur-1);
                       fdev:=fdev+(p^.d2-freq)*
                              (p^.d2-freq)/(dur-1);
                       p:=p^.next
                    end;
                 adev:=sqrt(adev);
                 fdev:=sqrt(fdev);
              end
     end
end;

procedure readdata(var data:a2;var count:integer);
var i,datum:integer;
begin
   i:=1;
   read(datum);
   while datum<>999 do
      begin
         data[i]:=datum;
         i:=i+1;
```

```
            read(datum)
         end;
      count:=i-1
   end;

procedure scan(var call:r1; var  ata:a2;
         count:integer;var ncall:  nteger);
const z = 255   ;
      y = 0     ;
var    i       : integer;
      first : boolean;
begin
   i:=1;
   ncall:=0;
   first:=true;
   while i <= (count-1) do
     begin
       if   (data[i]=z) and (data[i+1]=z)
         then
           begin
             ncall:=ncall+1;
             with call[ncall] do
              begin
                if first
                  then first:=false
                  else
                     with call[ncall-1] do
                          begin
                            ampl:=ampl/dur;
                            freq:=freq/dur;
                            stan_dev(head,call,ncall-1)
                          end;
                dur:=1;
                time:=data[i+5];
                n1:=data[i+6];
                n2:=data[i+7];
                ampl:=n1;
                freq:=n2;
                new(tail);
                new(head);
                tail:=nil;
                addqueue(call,ncall,n1,n2);
                i:=i+8;
              end
           end
         else
          begin
            with call[ncall] do
             begin
               dur:=dur+1;
               n1:=data[i];
               n2:=data[i+1];
               ampl:=ampl+n1;
               freq:=freq+n2;
               addqueue(call,ncall,n1,n2);
               i:=i+2
             end
          end
     end;
  with call[ncall] do
         begin
            ampl:=ampl/dur;
            freq:=freq/dur;
            stan_dev(head,call,ncall)
         end;
end;

procedure header(time_date, rat info s1);

begin
   page;
   writeln;
   writeln;
   writeln(' ',time_date:30,' ' 10,rat info 30);
   writeln;
   writeln;
   writeln(' call No.  interval   duration   mean ',
        'ampl   mean freq   sdev ampl   sdev freq');
   writeln
end;


begin(*  m a i n    p r o g r a m    *)
    min_level:=10;
    criterion:=similar;
    reset(term_in,    'file=*msource* interactive');
    rewrite(term_out, 'file=*msink*');
    for i:=1 to 5 do
        writeln;
    write(' ||||||||||||||||||||||||||||||||||||| ||||||||');
    writeln(' ||||||||||||||||||||||||||||||||||||');
    write('    V O C A L I Z A T I O N   D A T A  |||');
    writeln(' ||||||||| |||||||||||||||');
    writeln;
    readdata( data,count);
    scan(call,data,count,ncall);
    writeln(term_out,' e ter time and date of run ',
                  '  (maximum 30 char )');
    readln(te m_in,time_date:30);
    writeln(term_out,'  enter information about rat',
                  '  (maximum 30 char )');
    readln(term_in,rat_info:30);
    writeln(term_out,'  enter duration and speed of run');
    readln(term_in,run_dura,run_vel);
    writeln(term_out,'  enter ease of running ');
    writeln(term_out,'on a scale ',
    'from 1 (hesitant runner) to 10 (olympic marathoner)');
    readln(term_in,ease);
    writeln(' ':9,time_date 30,' ' 10,rat info:20);
    for i:=1 to 2 do
        writeln;
    writeln(' ':10,'r u n    p a r a m e t e r s');
    writeln;
    writeln(' ':20,'treadmill speed = ',run_vel 4,' m/min');
    writeln(' ':20,'treadmill grade =   10 degrees');
    writeln(' ':20,'duration        = ',run_dura 4,' min');
    writeln(' ':20,'number of calls = ',ncall 4);
    writeln;
    writeln;
    writeln(' ':10,'p e r f o r m a n c e   i n d e x');
```

```
    writeln;
    for j:=1 to 2 do
        begin
          write(' ':10);
          for i:=1 to 6*ease+1 do
              write('*');
          writeln
        end;
    write('+':11);
    for i:=1 to 10 do
        write('-----+');
    writeln;
    write('|':11);
    for i:=1 to 10 do
        write('|':6);
    writeln;
    write('0':11);
    for i:=1 to 10 do
        write(i:6);
    writeln;
    writeln(' ':10,'poor     ',' ':43,'excellent');
    for i:=1 to ncall do
        begin
          if (i-1) mod 50 = 0
             then header(time_date,rat_info);
          with call[i] do
             writeln(i:8,time:10,dur:11,ampl:12:4,
                freq:12:4,adev:12:4,fdev:12:4);
        {view_q(call[i].head)}
        end;
    writeln;
    writeln('-------------------------------------------------');
    writeln;
end
```

```
program surface;

   created      Feb. 4, 1985

   purpose -   to display the vocalization data
               in terms of a three-dimensional
               plot
                    x axis : amplitude
                    y axis : frequency
                    z axis : number of calls

               the program links up to a fortran
               graphics package entitled DISSPLA

   input --    digital ultrasound recorder output
               (unit=scards)

   output  -   surface plot(unit=*espp*)
                                                   )

type  a1 = array[1..128,1..128] of shortreal;
      a2 = array[1..30000] of integer;
      cell = record
               amp,frq,num : integer;
             end;(record)
      r1=array[1..4200] of cell     ;
var   graph                          : a1    ;
      data                           : a2    ;
      call                           : r1    ;
      zmax                           : real  ;
      ease,n1,n2,max,
      min_level,run_dura,run_vel,
      ncall,i,j,k,l,m,n,count,newcall  : integer;

procedure DISSPL(VAR GRAPH:A1; VAR ZMAX:REAL);
         FORTRAN;

procedure readdata(var data:a2;var count:integer) ;
var i,datum:integer;
begin
   i:=1;
   read(datum);
   while datum<>999 do
      begin
         data[i]:=datum;
         i:=i+1;
         read(datum)
      end;
   count:=i-1
end;

procedure check(var graph:a1; var zmax:real);

var i,j        integer;

begin
  ncall:=0;
  for i:=1 to 128 do
     for j:=1 to 128 do
        if graph[i,j]>zmax
           then zmax:=graph[i,j]
end;

procedure scan(var graph:a1; var data:a2;
                                 count:integer);
const z = 255   ;
      y = 0     ;
var   i       : integer;
      search: boolean;
begin
   i:=1;
   while i <= (count-1) do
     begin
        if  (data[i]=z) and (data[i+1]=z)
           then i:=i+6;
        n1:=data[i] div 2 +1;
        n2:=data[i+1]+1;
        if n2>128
           then n2:=128;
        graph[n1,n2]:=graph[n1,n2]+1;
        i:=i+2
     end
end;

begin(*  m a i n    p r o g r a m    *)
   zmax:=0;
   for i:=1 to 128 do
       for j:=1 to 128 do
          graph[i,j]:=0;
   readdata( data,count);
   scan(graph,data,count);
   check(graph,zmax);
   for i:=1 to 40 do
      begin
         for j:=51 to 70 do
            write(graph[i,j]:3:0);
         writeln;
      end;
   DISSPL(GRAPH,ZMAX);
end.
```

```
C      PROGRAM DISSPL
C
C       CREATED      FEB  4, 1985
C       PURPOSE  - TO LINK A PASCAL PROGRAM THAT
C                  GENERATES A 3-D PLOT WITH A
C                  FORTRAN GRAPHICS PACKAGE
C
C       INPUT    - ARRAY CONTAINING THE VOCALIZATION
C                  'MAT' AND THE MAXIMUM VALUE IN THE
C                  ARRAY
C
@PROCESS SC(HEADIN)
      SUBROUTINE DISSPL(A,ZMAX)
      DIMENSION WORK(4000),A(128,128)
C      DO 10 I=1,20
C  10 WRITE(6,*) (IA(I,J),J=1,20)
      CALL DSPDEV('PLOTTER')
      CALL LRGBUF
      CALL PAGE(11.,10.)
      CALL AREA2D(10.,8.)
C      CALL SWISSM
C      CALL SHDCHR(90.,1.,.002,1)
C      CALL BASALF('L/CSTA')
C      CALL MIXALF('STAN')
      CALL HEADIN('VOCALIZATION DATA$',.50,1.5,1)
      CALL HWROT('AUTO')
      CALL HWSCAL('SCREEN')
      CALL X3NAME('FREQUENCY',50)
      CALL Y3NAME('AMPLITUDE',50)
      CALL Z3NAME('NUMBER OF OBSERVATIONS',50)
      CALL VOLM3D(15.,15.,15.)
      CALL VUABS(30.,30.,15.)
      CALL GRAF3D(0.,16.,128.,0.,32.,256.,0.,10.,ZMAX)
      CALL SURMAT(A,2,128,2,128,0)
      CALL ENDPL(0)
      CALL DONEPL
      STOP
      END
```

```
program grayplot;

{   created      Feb  4, 1984

   purpose    to output the amplitude and frequency
              information of the vocalizations in a
              two-dimensional gray plot

   input    - digital ultrasound recorder output
              (unit=scards)

   output   - gray plot (unit=*espp*)
                                                   )
program readdata;

type  a1 = array[1..256,1..256] of
                packed -32768..32767;
      a2 = array[1..30000] of integer;
var   gray                                    : a1     ;
      data                                    : a2     ;
      ease,n1,n2,zmax,
      min_level,run_dura,run_vel,
      ncall,i,j,k,l,m,n,count,newcall)   : integer;


procedure IMPLOT(var gray:a1);
          FORTRAN;


procedure readdata(var data:a2;var count:integer) ;
var i,datum:integer;
begin
   i:=1;
   read(datum);
   while datum<>999 do
     begin
       data[i]:=datum;
       i:=i+1;
       read(datum)
     end;
   count:=i-1
end;


procedure scan(var gray:a1);
const z = 255          ;
      y = 0            ;
var   i,d1,d2 : integer;
      search    boolean;
begin
   i:=1;
   while i <= (count-1) do
     begin
       if  (data[i]=z) and (data[i+1]=z)
           then i:=i+6;
       n1:=data[i];
       n2:=data[i+1];
       if gray[n2+1,n1+1]=255
          then gray[n2+1,n1+1]:=0;
           i:=i+2
       end
end;


begin(*  m a i n    p r o g r a m     *)
    zmax:=0;
    for i:=1 to 256 do
        for j:=1 to 256 do
            gray[i,j]:=255;
    readdata( data,count);
    scan(gray);
    for i:=1 to 15 do
        for j:=1 to 256 do
            begin
              if gray[16*i,j]<>0
                 then gray[16*i,j]:=220;
              if gray[j,16*i]<>0
                 then gray[j,16*i]:=220;
            end;
    for j:=2 to 255 do
        begin
           gray[2,j]:=0;
           gray[255,j]:=0;
           gray[j,2]:=0;
           gray[j,255]:=0;
        end;
    IMPLOT(gray);
end.
```

```
program analysis;

     created      Jan 29, 1984

   purpose -   to analyze the rodent vocalizations
               in terms of time and frequency
               intervals

   input   -   digital ultrasound recorder output
               (unit=scards)

   output  -   table of vocalizations in terms of
               time and frequency intervals
               (unit=sprint)
                                                  )

type  a1 = array[1..128,1..128] of integer;
      a2 = array[1..40000] of integer          ;
      a3 = packed array[1..20] of integer;
      a4 = array[1..20] of a3                  ;
      cell = record
               amp,frq,num : integer;
             end;(record)
      r1=array[1..4200] of cell       ;
var   graph                                 : a1    ;
      data                                  : a2    ;
      f_hist,hist,coltot                    : a3    ;
      f_hist1                               : a4    ;
      cell                                  : r1    ;
      zmax                                  : real  ;
      ease,n1,n2,max,total,total1,
      min_level,run_dura,run_vel,f1,f2,
      ncall,i,j,k,l,m,n,count,newcall     : integer;

procedure readdata(var data:a2;var count:integer) ;
var i,datum:integer;
begin
   i:=1;
   read(datum);
   while datum<>999 do
      begin
         data[i]:=datum;
         i:=i+1;
         read(datum)
      end;
   count:=i-1
end;

function min(n,m integer):integer;

begin
   n:=n div 10;
   if n<m
      then min:=n
      else min:=m
end;

procedure scan(var f_hist:a3; var f_hist1:a4; var data:a2;
                              count:integer; var ncall:integer);
const z = 255   ;
      y = 0     ;
var   i,j    : integer;
      search: boolean;
begin
   i:=1;
   while i <= (count-1) do
     begin
        if  (data[i]=z) and (data[i+1]=z)
            then
              begin
                 i:=i+6;
                 j:=data[i-1]+1;
              end;
        if data[i+1]>99
           then n2:=6
           else n2:=data[i+1] div 20 +1;
        f_hist1[j,n2]:=f_hist1[j,n2]+1;
        i:=i+2
     end;
   ncall:=j;
   writeln('  number of time intervals = ',ncall:2);
end;


begin(*  m a i n    p r o g r a m   *)
     max:=0;
     for i:=1 to 6 do
          begin
            for j:=1 to 20 do
                f_hist1[j,i]:=0;
            f_hist[i]:=0;
          end;
     readdata( data,count);
     scan(f_hist,f_hist1,data,count,ncall);
     total1:=0;
     for i:=1 to ncall do
         begin
           k:=0;
           for j:=1 to 6 do
               k:=k+f_hist1[i,j];
           coltot[i]:=k;
           total1:=total1+k;
         end;
     if ncall >3
        then
          begin
            if ncall < 6
               then
                 begin
                   f1:=2;
                   f2:=ncall-1;
                 end
               else
                 begin
                   f1:=3;
                   f2:=ncall-2;
                 end;
```

```
for i:=1 to 6 do
    begin
      if f1=3
         then f_hist1[1,i]:=f_hist1[1,i]+
                            f_hist1[2,i];
      for j:=f1+1 to f2 do
           f_hist1[f1,i]:=f_hist1[f1,i]
                            +f_hist1[j,i];
      if ncall>5 then
           f_hist1[f2+1,i]:=f_hist1[f2+1,i]
                            +f_hist1[ncall,i];
      f_hist1[2,i]:=f_hist1[f1,i];
      f_hist1[3,i]:=f_hist1[f2+1,i];
    end;
  if ncall>5
     then
       begin
         coltot[1]:=coltot[1]+coltot[2];
         coltot[2]:=coltot[f1];
         for i:=f1+1 to f2 do
              coltot[2]:=coltot[2]+coltot[i];
         coltot[3]:=coltot[ncall-1]+coltot[ncall];
       end
     else
       begin
         for i:=f1+1 to f2 do
              coltot[2]:=coltot[2]+coltot[i];
         coltot[3]:=coltot[ncall];
       end;
end;
for j:=1 to 3 do
     begin
       for i:=1 to 6 do
            write(f_hist1[j,i]/coltot[j]*100:6:1);
       writeln
     end
end
```

```
program cell;

   created      March 10, 1985

   purpose :-  to format the digital ultrasound
               recorder output in such a manner
               that hierarchical log-linear
               analysis can be applied

   input    -  digital ultrasound recorder output
               (unit=scards)

   output   -  cell no., number of cell occurrences
               (unit=sprint)


type  a1=array[1..10]    of integer;
      a2=array[1..30000] of integer;
      ptr  = @node;
      cell = record
               head,tail   : ptr     ;
               time,dur    : integer;
               ampl,freq,
               adev,fdev   : real    ;
             end;{record}
      node = record
               d1,d2:integer;
               next:ptr;
             end;{record}
      qtr   = @node1;
      node1 = record
                store:a1;
                prv,nxt:qtr;
              end; {record}
      r1=array[1..1200] of cell      ;
      e1=(similar,noise_check)       ;
      s1=packed array[1..30] of char;
      s2=packed array[1..20] of char;
var   data                          : a2     ;
      call                          : r1     ;
      criterion                     : e1     ;
      time_date                     : s1     ;
      rat_info                      : s2     ;
      term_in,term_out              : text   ;
      qhead,q1,q2,q3                : qtr    ;
      ease,n1,n2,
      min_level,run_dura,run_vel,
      ncall,i,j,k,l,m,n,count,newcall : integer;

procedure addqueue(var call:r1; i,n1,n2:integer);

var p:ptr;
begin
  with call[i] do
    begin
      new(p);
      p@.d1:=n1;
      p@.d2:=n2;
      p@.next:=nil;
      if tail=nil
        then
          begin
            tail:=p;
            head:=p;
          end
        else
          begin
            tail@.next:=p;
            tail:=p;
          end
    end
end;

procedure view_q(p1:ptr);

var p:ptr;
begin
  p:=p1;
  while p<>nil do
    begin
      write(p@.d1:4,p@.d2:4);
      p:=p@.next
    end;
  writeln;
  writeln
end;

procedure stan_dev(p1:ptr; var call:r1; i:integer);

var p:ptr;
begin
  with call[i] do
    begin
      adev:=0.0;
      fdev:=0.0;
      if dur <> 1
        then
          begin
            p:=p1;
            while p<>nil do
              begin
                adev:=adev+(p@.d1-ampl)*
                        (p@.d1-ampl)/(dur-1);
                fdev:=fdev+(p@.d2-freq)*
                        (p@.d2-freq)/(dur-1);
                p:=p@.next
              end;
            adev:=sqrt(adev);
            fdev:=sqrt(fdev);
          end
    end
end;

procedure readdata(var data:a2;var count:integer);
var i,datum:integer;
begin
  i:=1;
  read(datum);
  while datum<>999 do
```

```
      begin
         data[i]:=datum;
         i:=i+1;
         read(datum)
      end;
   count:=i-1
end;

procedure scan(var call:r1; var data:a2;
        count:integer;var ncall:integer);
const z = 255   ;
      y = 0     ;
var   i      : integer;
      first  : boolean;
begin
   i:=1;
   ncall:=0;
   first:=true;
   while i <= (count-1) do
     begin
        if  (data[i]=z) and (data[i+1]=z)
          then
           begin
              ncall:=ncall+1;
              with call[ncall] do
                begin
                  if first
                     then first:=false
                     else
                        with call[ncall-1] do
                           begin
                              ampl:=ampl/dur;
                              freq:=freq/dur;
                              stan_dev(head,call,ncall-1)
                           end;
                  dur:=1;
                  time:=data[i+5];
                  n1:=data[i+6];
                  n2:=data[i+7];
                  ampl:=n1;
                  freq:=n2;
                  new(tail);
                  new(head);
                  tail:=nil;
                  addqueue(call,ncall,n1,n2);
                  i:=i+8;
                end
           end
        else
         begin
            with call[ncall] do
              begin
                 dur:=dur+1;
                 n1:=data[i];
                 n2:=data[i+1];
                 ampl:=ampl+n1;
                 freq:=freq+n2;
                 addqueue(call,ncall,n1,n2);
                 i:=i+2
              end
               end
         end;
    with call[ncall] do
      begin
         ampl:=ampl/dur;
         freq:=freq/dur;
         stan_dev(head,call,ncall)
      end;
end;

procedure header(time_date:s1; rat_info:s2);

begin
   page;
   writeln;
   writeln;
   writeln(' ',time_date:30,' ':10,rat_info:20);
   writeln;
   writeln;
   writeln(' call No   interval   duration   mean ',
          'ampl   mean freq   sdev ampl   sdev freq');
   writeln
end;

function identical(n:integer; k1,k2:a1):boolean;

var i    : integer;
    test : boolean;

begin
  test:=true;
  i:=1;
  while test and (i<=n) do
    if k1[i]<>k2[i]
       then test:=false
       else i:=i+1;
  identical:=test;
end;

begin(*  m a i n    p r o g r a m     *)
     min_level:=10;
     criterion:=similar;
     reset(term_in,    'file=*msource* interactive');
     rewrite(term_out, 'file=*msink*');
     readdata( data,count);
     scan(call,data,count,ncall);
     new(q1);
     qhead:=q1;
     qhead^.prv:=nil;
     writeln(' number of calls = ',ncall:4);
     for i:=1 to ncall do
            with call[i] do
              begin
                qie.store[1]:=time+1;
                if dur>40
                   then qie.store[2]:=4
                   else qie.store[2]:=(dur div 10)+1;
                if freq<50
                   then qie.store[4]:=1
                   else qie.store[4]:=2;
```

```
            q1^.store[3]:=( round(ampl) div 64)+1;
          ( q1^.store[4]:=( round(freq) div 8)+1; )
            q1^.store[5]:=1;
            if i<>ncall
              then
                begin
                  new(q2);
                  q1^.nxt:=q2;
                  q2^.prv:=q1;
                  q1:=q2;
                end
              else q1^.nxt:=nil;
          end;
        {view_q(call[i].head)}
    q1:=qhead;
    while q1<>nil do
      begin
        q2:=q1^.nxt;
        while q2<>nil do
          if identical(4,q1^.store,q2^.store)
            then
              begin
                q1^.store[5]:=q1^.store[5]+1;
                q3:=q2^.prv;
                q3^.nxt:=q2^.nxt;
                q2:=q2^.nxt;
                if q2<>nil
                  then q2^.prv:=q3;
              end
            else q2:=q2^.nxt;
        q1:=q1^.nxt;
      end;
    q1:=qhead;
    j:=1;
    k:=0;
    while q1<>nil do
      begin
        write(j:4);
        j:=j+1;
        k:=k+q1^.store[5];
        for i:=1 to 5 do
            write(q1^.store[i]:4);
        writeln;
        q1:=q1^.nxt
      end;
    writeln(' ** check **    number of calls = ',k:4);
end
```

Appendix 4

SPSSx Routines

```
file handle test/name='-o'
data list  file=test record=1
 /1 num 6-9 inter 17-19 dura 27-30 ampl 34-42 (4) freq 46-54 (4)
   sd.amp 58-66 (4) sd.frq 70-78 (4)
variable labels
   num        'call number          '
   inter      'call interval        '
   dura       'call duration        '
   ampl       'amplitude of call    '
   freq       'frequency of call    '
   sd.amp     'stan dev of call ampl '
   sd.frq     'stan dev of call freq '
pearson corr inter dura ampl freq sd.amp sd.frq
options 3
statistics 1,2
finish
```

```
file handle testlog/name='-4'
data list  file=testlog record=1
 /1  inter 6-9 dura 10-13 ampl 14-17 freq 18-21 num 22-25
variable labels
   inter 'time of call          '
   dura  'duration  of call     '
   ampl  'amplitude of call     '
   freq  'frequency of call     '
   num   'number of calls in cell'
weight by num
hiloglinear    inter(1,15) dura  ampl (1,4) freq (1,2)    /
               print = association  /
               maxorder=4           /
               design               /
```