THE "DISTRESS" CRY OF INFANT DEERMICE, PEROMYSCUS: PHYSICAL CHARACTERISTICS, SPECIFIC DIFFERENCES, SOCIAL FUNCTION.

DISSERTATION FOR THE DEGREE OF PH. D.

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THESIS





This is to certify that the

thesis entitled

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ABSTRACT

THE "DISTRESS" CRY OF INFANT DEERMICE, <u>PEROMYSCUS</u>: PHYSICAL CHARACTERISTICS, SPECIFIC DIFFERENCES,

SOCIAL FUNCTION.

by Jane Netting Huff

Isolated <u>Peromyscus</u> pups vocalize with rapid sets of ultrasonic squeaks. These sounds were recorded from pups of 8 different taxa in order to quantify speciesspecific characteristics and parameters showing developmental changes. The behavioral responses of adult female <u>Peromyscus</u> to recorded squeaks were tested to clarify the function of the calls.

Pups of <u>Peromyscus maniculatus bairdi</u>, <u>P.m.gracilis</u>, <u>P.polionotus</u>, and <u>P.leucopus</u>, were recorded from day 1 after birth until the average age of eye-opening. Pups of <u>P.californicus</u>, <u>P.melanophrys</u>, <u>P.m.nebracensis</u>, and <u>P.</u> difficulis were recorded when available.

Statistical analyses showed significant interspecific differences in the number of squeaks in a group of squeaks (set), the duration of individual squeaks, and in basic frequencies. The <u>P.maniculatus</u> subspecies showed similar mean basic frequencies (bairdi - 22.6 kHz,



<u>gracilis</u> - 22.9 kHz, <u>nebracensis</u> - 23.7 kHz) as did <u>P</u>. <u>melanophrys</u> (22.8 kHz). Mean basic frequencies for <u>P</u>. <u>leucopus</u> and <u>P.polionotus</u> were higher (26.7 kHz and 31.4 kHz) while those for <u>P.californicus</u> and <u>P.difficilis</u> were lower (18.6 kHz and 19.9 kHz). These frequencies tended to rise with age.

Squeak duration differed significantly between the taxa from <u>P.polionotus</u> (0.13 sec.) to <u>P.m.gracilis</u> and <u>P.</u> <u>californicus</u> (0.18 sec.). Set duration did not differ significantly across the groups. The number of squeaks per set for <u>P.m.bairdi</u> and <u>P.polionotus</u> (3.70) was significantly different from the mean for <u>P.m.gracilis</u> (4.05) and <u>P.leucopus</u> (3.15). These temporal parameters all decreased significantly in mean value over age.

Comparison of oscilloscope films and sonagrams demonstrated species-specific patterns of frequency and intensity modulation. Each of the taxa studied differed from the others in at least one quantifiable parameter and in its modulation patterns. These calls also showed characteristics which make sounds easy to locate, such as high frequencies, frequency and intensity modulation, redundancy, and sharp rise and fall.

The hypothesis that these calls might affect the behavior of a mother mouse, (1) by increasing her activity and stimulating her to search for a "pup-in-distress"; (2) by enabling her to efficiently locate a calling pup; and (3) by providing sufficient long-distance information

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for preferential responses to species, was tested with groups of mice in two modified open-field boxes. <u>P.m.</u> <u>bairdi</u> mothers were tested in a two-way box, for differential activation and preference for recorded squeaks of <u>P.m.bairdi</u> pups versus mechanical clicks and white noise. In terms of numbers of responses, correct choices, and distances traveled towards the mouse squeaks, the test and control groups preferred the squeaks to clicks or noise. Similar groups of <u>P.m.bairdi</u> and <u>P.leucopus</u> mothers preferred <u>leucopus</u> squeaks to white noise. But those test groups given a choice between squeaks of the two species showed minimal discrimination.

<u>P.m.bairdi</u> mothers showed accurate location of the source of squeaks from recordings of live pups in a fanshaped test box. Responses to recordings were faster and more accurate than to live if first choices alone were considered but a mean of three choices showed no significant differences between responses to live pups and recordings.

These data are interpreted to mean that the squeaking of a pup dropped by a mother mouse moving a nest or fleeing a disturbance is both a necessary and sufficient factor in ensuring retrieval and survival. The sounds alert the mother mouse to the situation, allow her to locate the calling pup efficiently, thus reducing risks of searching in the open, and possibly even permit her to respond preferentially to her own species. The ontogenetic changes in the characteristics of the squeaks correlate with the decreasing amounts of maternal behaviors, possibly because of reduced stimulus effectiveness. Differences in the squeaks occur in temporal and physical parameters across ages and between species in Peromyscus.

THE "DISTRESS" CRY OF INFANT DEERMICE, <u>PEROMYSCUS</u>: PHYSICAL CHARACTERISTICS, SPECIFIC DIFFERENCES, SOCIAL FUNCTION.

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(Fisher, 1969)

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INTRODUCTION

The distress cries of an infant mammal usually bring a warm, nourishing parent to carry it back to a secure nest. This et-epimiletic/epimiletic behavior sequence is frequently observed but seldom considered as more than an example of maternal behavior. Most studies of retrieving have concentrated on the physical and hormonal aspects of the behavior of the mother. However, the behavior of the mother is related to the behavior of the infant by the communication of information. The helpless infant transmits a message through some channel by means of a signal and that signal is received, decoded, and acted upon by a receiver, the mother. In the following study I looked at the behaviors of crying infant/retrieving mother as a communication system and by analyzing the characteristics of the signal and the response of the receiver I attempted to decipher the code.

As the original impetus for this study grew out of a general interest in mammalian sounds it seemed appropriate to use rodents as experimental animals, since their retrieving behavior has been well analysed. Rodent sounds, however, have only recently become susceptible to analysis as equipment has been developed to cope with their very high frequencies.

In terms of communication theory five channels are available for transmitting information such as the distressed state of a rodent pup to its parent; touch, taste, olfaction, vision and sound. Although each has intrinsic advantages and disadvantages, sound appears to be the preferred channel for distance communication among rodents. Sounds can be directly tuned to the ears of the receiver, they do not linger on the air, and they can be easy or difficult to locate, depending on characteristics of the sound. Another peculiar advantage of the sound channel is that signals can be produced without ceasing any ongoing activity and they can be received by a moving or even a sleeping animal. Distress signals emitted by infant mammals are usually sounds.

Sounds are conveniently the signals most amenable to electronic analysis and the most easily separated from "noisy" surroundings. Echolocation signals of numerous mamals have been studied in detail but the other sounds of other mammals such as rodents, have only received incidental attention. In part this has been due to the generally nocturnal and secretive habits of rodents and in part to the fact that many rodent calls are above the range of human hearing, being partly or completely ultrasonic (i.e., above 20 kHz).

Deermice (<u>Peromyscus</u>) are typical of rodents in that they make chirps and squeaks with both sonic and ultrasonic components. Their nocturnal, retiring habits make

sound an important communication channel for distance signalling. Because their behavior patterns are well known (Eisenberg, 1962; Horner, 1940; King, 1968) these mice make excellent subjects for a study of their sounds as behavioral stimuli in communication systems.

Although deermice are known to produce sounds when fighting, courting, when disturbed, excited or frustrated, and in other situations of unknown significance (Eisenberg, 1962; King, 1968; personal observations) few of these sounds have been recorded and none analysed. As adult mice are often inhibited from vocalizing by the necessary restrictions of recording situations the only mouse sounds which have so far been examined in any detail are those which can be elicited involuntarily from either adults or young.

Berlin (1966) found that adult pain squeaks to shock had major intensities at 50 and 90 kHz. My own preliminary observations and published data from Housknecht (1968), King (1963), and Hart and King (1966) showed wide, and variable ranges of maximum sound energy for the infant distress cries of several species and subspecies of <u>Pero-</u> <u>myscus</u>. The present study concentrated on infant distress cries because they can be reliably elicited and because they form part of a communication system in a way that the pain squeaks do not. Preliminary studies showed that these cries varied across species and possibly ages in a visible and quantifiable manner, they are reliably reproduced by

pups isolated momentarily from their nests, and they elicit a predictable response from adult mice, i.e., retrieving.

Despite the small amount known about the physical characteristics of the sounds, several studies have provided data on the hearing acuity of mice in this genus. Dice and Barto (1952) found ear-twitch responses to sounds in certain species of <u>Peromyscus</u> up to 100 kHz. Ralls (1967) found evoked auditory potentials to tones of 100 kHz in <u>P.leucopus</u> and <u>P.boylei</u>. It is therefore apparent that the hearing of deermice is quite adequate for all of the frequencies they are now known to produce.

Rowell (1960) defines retrieving as "the response of the mother to the situation 'young outside the nest'." Rats, mice, hamsters and many other rodents respond to distress squeaks by approaching a squeaking infant, grasping it firmly in their teeth, and carrying or dragging it back to the nest. Retrieving can be elicited from virgin females and males, as well as lactating females, so it is not strictly dependent on the hormonal state of the adult, but rather on the complex of stimuli presented by the infant (Beach & Jaynes, 1956a, 1956b; Bell, et al, 1971; Horner, 1947; Beniest-Noirot, 1958; Richards, 1967; Rowell, **1960).** Anosmic rats and vibrissae-less or vibrissaedenervated rats will still retrieve (Beach & Jaynes, 1956a). However, Bell, et al (1971) found that during recording sessions when no vocalizations were detected, mother housemice neither retrieved infants nor groomed them. Sounds are

mentioned by Noirot and others as the factor which may first attract a mother's attention (Beniest-Noirot, 1958; Noirot & Pye, 1969; Zippelius & Schleidt, 1956).

Rodent parents appear to recognize their own young on the basis of the various stimuli emitted by the infant even though these stimuli change as the infant grows (Beach & Jaynes, 1956a; Noirot, 1960; Richards, 1967; Rowell, 1960). Hamsters attack and eat strange pups depending on the age but retrieve their own (Rowell, 1960). One major component cue for this recognition is odor, for Beach and Jaynes (1956a) concluded that their experimental rats could learn to recognize their own young by odor, after having retrieved only one. Sound is proably sufficient for distance identification, at least of species, since while prairie deermice (<u>Peromyscus maniculatus</u>) do not respond to the calls of infant California deermice (<u>P.californicus</u>) they readily search for calling pups of their own species (personal observations).

With the above considerations in mind I designed this study to explore the nature of the squeaks of isolated mouse pups, and to examine the behavioral responses of mother mice to these signals. In the first part of the study the distress cries of pups of several species of <u>Peromyscus</u> were recorded and analysed with an oscilloscope and sound spectrograph. I hypothesized that if distress squeaks of these mice have evolved as specific signals alerting an adult to the plight of the squeaker and

attracting and directing her search, as quickly as possible, then the squeaks should show typical characteristics of easily localizable sounds. I expected that the squeaks would have sharp rise and fall times, pure tones with short wave lengths and maximal sound energy at levels best heard by adult mice and intensity modulation. These characteristics would enable the relevant hearers to locate the sounds by means of time differences, phase differences and differential sound levels at their two ears. For information transfer with minimal error the sounds should incorporate some maximum amount of redundancy. As the mouse pup opens its ears and hears its own sounds all these characteristics could be refined to some species typical pattern and therefore show a reduction in variability with age.

Preliminary analyses indicated that intensity modulation could only be compared visually from oscilloscope films but not measured as it was impossible to maintain a stable baseline. Frequencies were precisely measured with the sound spectrograph and frequency modulations were depicted by the sonagraph. Temporal characteristics of number of squeaks in a set, and the duration of squeaks and sets could be measured on the oscilloscope films. Other characteristics of the squeaks such as onsets and offsets and harmonic structures were clearly visible on the sonagrams.

In the second part of this study I observed the behavior of lactating female deermice in modified open field

test boxes when they heard recorded and live distress calls. Studies on retrieving in mice indicate, in general, that lactating females respond very quickly to squeaking pups but not to quiet ones (King, 1963; Noirot, 1958; Sewell, 1970). If the sound alone is a sufficient stimulus to a mouse to retrieve, then I hypothesized that appropriately recorded sounds ought to stimulate retrieving even in the absense of the other stimuli from a living pup. If the stimulus is strong enough to act in a natural environment under a situation of stress, as for instance where a mother is moving a nest disturbed in daylight, then it ought to be sufficiently strong to lure a mother mouse out into an open arena.

In two tests designed to test the hypothesis that the squeaks alone were sufficient and necessary to stimulate mother mice to search and enable them to discriminate, females with litters were housed in the center of a long, sound-choice box (SCB) which had a speaker at either end. Recorded squeaks and control sounds were played alternately from opposite speakers and the approach responses, or lack of approach, of the mothers were recorded. Responses to squeaks of conspecifics were compared to responses to squeaks of pups of other species or mechanical sounds.

In a third test designed to measure the localization abilities of the mice lactating females were allowed to enter a fan-shaped test box at the center when sounds were played from speakers randomly located on the periphery of

the fan. The accuracy of their responses was scored and responses to recorded squeaks were compared with responses to squeaks from live pups.

By means of this two-pronged approach, first, the examination of the characteristics of the signals, and then measurement of the responses of the relevant receiver I attempted to define the possible and probable information carried in the message of the distress calls of the infant deermouse. I suggest (anthropomorphically) that the squeaking pup is not just shouting "help" but something more like "Here I am, a 6 day old <u>Peromyscus maniculatus bairdi</u> baby, out of my warm nest, come and get me, right here!"

CHAPTER 2

Literature Review: The Physical and Functional Correlates of Ultrasound in Young Rodents

While it may be intrinsically obvious to the nonethologist that mammalian mothers usually rush to the "rescue" of their helpless babies, the inquiring ethologist cannot accept such a truism unexamined. A host of questions is contained in this seemingly simple activity. As this study was designed to answer some of those questions, for one species, a review of the pertinent literature is appropriate.

In this review I will attempt to establish a background for subsequent discussions of several of the most important questions. Considering rodents, in general, and <u>Peromyscus</u>, in particular, what do we know about the impetus to the rescue? What role does the helpless infant play in regard to the mother's behavior? Are the "distress" cries of the infants necessary stimuli to the maternal search?; do they help the mother locate the infant? What other information is contained in the cries, if any? Are there other functions possible for the calls or other cues for the retrieving mother? What prompts the pup to call? What are the characteristics of the calls and how do they

vary ontogenetically and phylogenetically? Finally, of what ecological (and selective) advantage is such a communication system to the animals involved?

In the past there have been two general experimental approaches to the "baby cries - mother comes" situation. Infant rodent calls have been analyzed in terms of rate and intensity and the variations of these related to age, handling, or temperature, and described in terms of frequency. Meanwhile, the stimuli eliciting various maternal behaviors have been examined. In the experiments and analyses described here I attempted to consider the communication system as a whole, looking at the behavior of both mother and pup and at their interaction. This literature survey will reflect this attempt at synthesis.

Sonic and Ultrasonic Communication in Rodents:

The audible squeaks and twitterings of small rodents have long caught the ears of listening naturalists. Unusually long or high squeaks from "singing" mice were compared to bird calls and sparked imaginative discussions as to their mode of production and function. (Dice, 1932).

Studies of ultrasonic echolocation signals in bats prompted searches for sounds with similar function in other small mammals (Gould, <u>et al.</u>, 1964; Kahmann & Ostermann, 1951). In the case of rodents no specific echolocation sounds have been demonstrated, although incidental sounds, snufflings, footsteps and other sounds may well aid in

rodent navigation in the dark (Anderson, 1954; Kahmann & Ostermann, 1951; Rosenzweig, Riley & Krech, 1955).

Sounds used for communication have been demonstrated in almost all rodents (Tembrock, 1963, 1968) but precise physical and functional analyses are rare. In part this is due to the enormous technical difficulties inherent in recording very high frequency sounds from easily disturbed animals. Within the last five years or so, advances in acoustic recording equipment has made it more possible, although expensive, to do detailed analyses of sounds of all frequencies produced by rodents.

Studies of rodent sounds have generally been of two types, those done as part of an ecological or behavioral study of the animal, or those in which the frequency ranges of single sounds of many diverse rodents have been examined. Most of the data have been obtained from diurnal, social rodents.

The sounds are usually differentiated in terms of the environmental situation in which they occur, the presumed physiological state of the animal, and the subjective appearance of the sound to the human observer. (For example, see Balph & Balph, ground squirrels, 1966; Fitch, 1948, ground squirrels; King, 1955, prairie dogs; Rood, 1972, cavies; Ruffer, 1966, grasshopper mice; Waring, 1966, marmots.)

Particularly in field studies, the social function

of a particular sound may be unclear. Listening for "mating" calls, or "abandonment" cries may be inappropriate for studies of rodent communication where one sound may be uttered in several seemingly different social situations (Brooks & Banks, 1973). When considered in terms of information sent out by the caller, relevant to its individual situation rather than to the social situation as a whole, calls can be grouped into two functional systems (Tembrock, 1968).

Dispersal Sounds; Contact Calls:

The first group may be considered dispersal sounds, serving to increase the distance between sender and receiver in agonistic and defensive situations. The second group of sounds are used primarily between conspecifics as selfadvertisement and contact-soliciting calls. In the simplest form these calls carry information about the species, sex, age, and location of the emitter. Reception of this information may reduce the impetus to flight or fight on the part of the relevant hearer or elicit its approach to the caller.

Both vocal and non-vocal (that is not produced by the vocal cords) sounds may fit in either category and function in audible communication. Unvoiced sounds, however, are predominately "white" noise, often involuntary warning sounds. While the tempo of paw-tapping in gerbils, for example, can be varied with the intensity of the situation, the frequencies cannot be modified and the repertoires are
extremely limited.

Voiced Sounds:

Voiced sounds are therefore, more interesting, having more complex codes and variations possible. Sound repertoires in rodents are not as extensive as those of primates, but sound categories may be more discrete and non-overlapping.

Vocalizations reported in marmots (Armitage, 1962; Lloyd, 1972; Waring, 1966), ground squirrels (Balph & Balph, 1966; Fitch, 1948), prairie dogs (King, 1955), squirrels (Zolley, 1971), laboratory rats (Anderson, 1954), and several genera of mice (Eisenberg, 1968; Layne, 1968; Ruffer, 1966; Sewell, 1968; Tembrock, 1968) function in social situations, a majority of the sounds being used in establishing or maintaining contact.

Observations of the bank vole (<u>Clethrionomys glareo-</u><u>lus</u>) convinced Schleidt (1948) that the two types of high squeaks they produced were used in communication. A "squeaking hiss" was heard in conflicts over food and a high, weak, squeak was described as a contact sound. In 1951, Schleidt further described the situations in which the bank vole produces high-frequency sounds. In the presence of females or near nest areas the males of this species gave short squeaks of about 15.5 kHz, possibly contact calls. The sounds of young mice were noted by Zippelius and Schleidt (1956) in Apodemus, Mus, and Microtus. Here the authors

noted a loud clicking, sucking sound with the greatest intensity between 5 and 10 kHz and some frequencies to 40 kHz. A squeak with components to 80 kHz was observed and this "abandonment" whistle caused the female to notice a calling infant and retrieve it. When the pups were picked up by the scruff of the neck, by the experimenter, another sound was noted, having components up to 100 kHz.

Houseknecht (1968) used sonagrams to analyze the vocalizations of adult <u>Mus, Peromyscus leucopus</u> and <u>Microtus pennsylvanicus</u>. Vocalizations were recorded in aggressive interactions in the laboratory. Most of the aggressive encounters with all 3 genera were accompanied by very short "squeals" or "chits." In non-contact threat situations, where the provenance of the sound could be determined, only the subordinate mice vocalized, possibly a defense/spacing cry. Sounds of the three genera roughly compared.

Barfield and Geyer (1972) found a sound emitted by the post-ejaculatory male rat with components at 22 kHz and a duration of about 1-3 seconds. Brooks and Banks (1973) in their exhaustive catalogue of lemming sounds found a similar male call. In both cases these appear to be "spacing" sounds (desist contact) similar to that heard from supine subordinate male rats. The rat sounds show sharp onsets and offsets characteristic of easily localizable sounds.

Brooks and Banks (1973) recorded and analysed 6 types of calls from lemmings (<u>Dicrostonyx groenlandicus</u>) in terms of behavior and acoustic characters. They found wide variation in the physical properties of the sounds and emphasized that the variation in social situations in which particular sounds were found show that emission of sounds is dependent on the internal physiological state of the animal and the external situation and not on a conscious attempt on the part of the animal to communicate information (Brooks & Banks, 1973).

Sales (1972) detected ultrasounds during aggressive interactions in lab rats, Apodemus, Acomys, Thamnomys, Arvicanthis, Praomys, Mus, Mesocricetus, Meriones, Gerbillus, Clethrionomys, Ligurus, Microtus and Sorex. This study grew out of the observation that the "distress" cries of young rats could be elicited after the usual cessation of squeaking at eye opening if the pups were handled. The handling often forced the older animals into submissive postures. Submissive adult rats produced long pulses (800-1600 msec.) having narrow bandwidths (less than 5 kHz) and attacks on these rats were reduced. Short pulses (3-65 msec.) with wide bandwidths (5-10 kHz) were highly correlated with escape behavior and aggressive acts. The other species of rodents observed showed similar patterns of ultrasound production during aggressive encounters.

Ultrasounds in Infant Rodents:

Ultrasounds of young rodents are easily elicited from

isolated pups and have been the focus of several studies. Sewell (1968) found ultrasounds reported for young of all species of myomorph rodents - usually having the function of inhibiting attacks and aiding the retrieval of the pups. Ultrasonic calls recorded from 5-day old <u>Apodemus</u> <u>sylvaticus</u> pups initiated retrieving responses in listening mothers (Sewell, 1968).

Clicking, sucking sounds reported in small rodent pups by Zippelius and Schleidt (1956) were also described by Hart and King (1966), and Eisenberg (1968) for <u>Peromyscus</u> pups. Personal observations agree with Eisenberg's indication that these sounds are physical concomitants of sucking, producing a sound when the pup continues to nurse after being removed from the nipple. Although these sounds are sometimes discussed as vocalizations they are non-vocal and of no discernible communicatory value.

Two vocalizations have been generally found in young rodents, a long, high sound, often called an "abandonment" or "distress" cry, and a shorter squeak resulting from rough handling, the "pain" cry. Eisenberg (1968) also mentions "comfort" sounds, described as "low, rapid peeps made by the neonate while the female grooms it." No other authors mention this sound.

Signal Characteristics:

The "distress" cries which are the main subject of

this dissertation have been described as being "barely audible, high-pitched squeaks" (Layne, 1968). More precise descriptions are given for various species of Peromyscus by Eisenberg (1968) and Hart and King (1966). Eisenberg found 2 types of distress cries in these mice; one, a low (fundamental about 3000 Hz) sound rising about 500 cycles and falling with sharp onsets and offsets, the other a high (fundamental approximately 25 kHz) sound with a similar pattern to the first but repeated rapidly. The first was found in Peromyscus maniculatus, P. crinitus, and P. californicus and the second was found in these mice but also in P. eremicus. These cries have complex harmonics and Eisenberg states that the greatest energy may be found above the fundamental, with the important communication sounds being ultrasonic.

Hart and King (1966) described the distress vocalizations of pups of 2 subspecies of <u>P</u>. <u>maniculatus</u> as being short pulses (0.1 - 0.2 sec.), in groups of 2 - 5, with dominant frequencies up to 26.5 kHz (<u>P.m.gracilis</u>). The lowest frequency band was not found to be the most intense but the greatest sound energy was usually in an upper band. Frequencies above 30 kHz were not measured.

At the time that I began this study (1968) the descriptions above were the most precise available. Comparisons of descriptions of the sounds within the genus <u>Peromyscus</u> and with other rodent genera were difficult,

and still are, because of a general lack of precision and standardization of terms such as "fundamental." Many authors appear to consider the fundamental to be simply the lowest frequency visible on the sonagram and often attribute an enormous frequency spread to it, for example, 300 Hz - 25,000 Hz (Eisenberg, 1968). Ladefoged (1962) defines the "fundamental frequency" as "the frequency of repetition of a complex wave" or "when a repetitive wave form is analysed into its component frequencies the fundamental is the highest common factor of the component frequencies." He goes on to state that "the component with this frequency may have a large amplitude,... a small amplitude, or...there may not be any component with this frequency." That frequency with the most energy may, instead, be called the "basic frequency" particularly when the wave form under consideration is a non-repetitive type which does not really have a fundamental frequency (Ladefoged, 1962). Some authors listing fundamentals admitted that these might not be of major communicatory importance, whereas others reported different frequency measures (as "low and high frequency limits of the dominant frequency"; Hart and King, 1966). Sonagrams provided a visible comparison but subtle intricacies of sound patterns do not reproduce well by this method and quantitative comparisons are impossible without equivalently calibrated machines.

Since 1966 the distress cries of white mice, white rats, hamsters, and lemmings have been described in detail

by Allin and Banks (1970), Brooks and Banks (1973), Noirot (1966b, 1968), Noirot and Pye (1968), Okon (1970a,b, 1971), Sewell (1968, 1970) and Smith (1972). Only Smith has dealt with Peromyscus.

Data reported by Smith (1972) are for <u>P</u>. maniculatus (unidentified subspecies) and describe an "audible" squeak of 15 - 200 msec. duration, consisting of 2 or 3, occasionally 4, constant frequency components; a fundamental and 2nd and 3rd harmonics. The fundamental at 8-29 kHz was usually the principle component. Pulses are described as rising by 5 kHz at the onset and falling by approximately the same amount at the end. "ultrasonic" pulses of 3-45 msec. duration, consisting of a single frequency component with a rapid downsweep were found in the 60-140 kHz range. Smith also found a low frequency (3 kHz) multi-component pain squeak.

Motivation for Signaling: Temperature.

Although Hart and King (1966) and Eisenberg (1968) had suggested cold stress as a motivational factor in signaling, no detailed studies of such factors appear until Okon (1970). When Hart and King used cold stress to elicit vocalizations from their <u>Peromyscus</u> they did not mention the exact temperature of the surroundings of the pup during recordings. They suggest that the development of homeothermy may be related to the cessation of squeaking at eye Opening in these mice.

Noirot and Pye (1969) found that ultrasound production by albino mouse pups showed a sharp drop in intensity and frequency on days 6 and 7 and suggested that this might correlate with the development of homeothermy. This drop was in direct contrast to the results of a study by Okon (1970a) who reported that albino mouse pups produced weak ultrasounds until day 5 or 6 and stronger responses thereafter. Okon (1970a,b; 1971) considers that the response examined by Noirot and Pye was due to another motivational factor, handling, as these authors picked up the pups, or rocked them in order to provoke squeaks.

Okon (1970a, 1971) studied the development of homeothermy in albino mice, rats and hamsters and the concurrent changes in ultrasounds. He found that albino mice are almost completely poikilothermic at low temperatures from day 1 until day 6. From about day 7 they show homeothermy at median temperatures (22°C) and at day 15 they are developing homeothermy at low temperatures (2-3°C). At about this time the pups stop producing ultrasounds when merely exposed to low temperatures. By day 19 or 20 no calling is elicited even with temperatures as low as 2-3°C. The acoustic response to cold is strongest from days 6-7 to day 12 and weak before day 6. Low ambient temperatures, in this study, led to increased rate and intensity of ultrasounds, although the very low temperatures had an inhibiting effect. With severe chilling, sounds cease altogether, along with breathing, after a time which depends on the age

of the pup.

Allin and Banks (1970) used cold stress (2°C and 20°C) to elicit sounds from albino rat pups. They found that after 8 days of age the call rate declined in a fashion significantly correlated with age and possibly with the development of homeothermy. Heat stress (40°C) was not found to elicit calling and neutral temperatures (35°C) elicited only infrequent calling.

There can no longer be any doubt that some of the ultrasonic (and sonic) calls of infant rodents are casually linked to the degree of cold stress acting on the pup. As the physiologic temperature regulation of the pup matures, cold stress may not be felt as soon, or as strongly, and the lesser stimulation is reflected in reduced intensity and rate of calling. At the age at which the homeothermic system is sufficiently adult to protect against all but the coldest temperatures, the pup is rarely stimulated to squeak. Coincidentally this is also the point at which the eyes usually open.

Motivation for Signaling: Handling.

Noirot (1970) suggests that 2 types of distress call exist; one emitted during cold-stress and one during handling. She postulates that the first call serves as a stimulus for retrieving and the second results in a cessation of parental activity, particularly attack.

Bell, Nitschke, Gorry and Zachman (1971) considered

the stress of handling to be a factor in the elicitation of squeaks from P. maniculatus bairdi. The authors removed pups from the nest, placed them in small compartments and kept them there for 3 minutes. After they were returned to the nest, recordings were made of the ultrasounds produced by these "handled" pups and non-handled (left in the nest) controls. No mention is made of whether the temperature in the box was maintained as a neutral temperature but the authors attribute significant differences in rate (number of signals per session) and higher frequencies with longer mean squeak duration, which they found in the ultrasounds produced by the experimentals, to the handling. However, sonagrams presented with the data do not make the frequency differences clear, as the sounds pictured for the "handled" mice resemble typical cold-stress cries.

Okon (1970b) does clearly differentiate the 2 types of signal. Handling by the observer, loss of balance, and actual retrieving by the mother, all discussed as "tactile stimuli" by Okon, evoked ultrasounds from albino mice. The loudest pulses were produced by pups from day 1 on, while at this age only weak signals are produced during temperature stress (Okon, 1970a). After day 6 pups are less likely to fall over and the tactile stimulus squeak is less often produced. Okon also describes a "pain" squeak to a pinch or tap on the tail which was found to be louder than the other squeaks, with frequencies ranging from 80-100 kHz

(1970b). Okon suggests for the youngest pups the greatest danger is from cannibalism, so these produce the "handling" call most loudly, inhibiting attack from adults. Older pups (after day 6) are more liable to move, or be moved, out of the nest and so squeak most loudly to cold-stress. In any particular situation the 2 types of call may be mixed (Noirot, 1972).

Smith (1972) differentiates between audible (defined as below 30 kHz) and ultrasonic calls in <u>Peromyscus</u>. From the mouse's "point of view," of course, audible versus ultrasonic is a spurious distinction. Neutral terms related to the function of the calls would have been more realistic. Whatever the name, Smith finds significantly different frequencies and loudness between the 2 types of call. The "audible" pulses are emitted in situations of cold stress while the ultrasonic pulses seem to be "handled" calls.

Phylogenetic Differences:

Eisenberg (1968) and Hart and King (1966) have so far been the only ones to compare sounds of different groups of <u>Peromyscus</u>. Eisenberg lists different frequencies and measurements of squeak duration for 4 species of Peromyscus (1968). The two types of "abandoned" cry discussed here may be cold-stress and handled cries although the differences are not very clear. Long (0.40-0.50 sec.), low (4-22 kHz) cries were typical of <u>P</u>. <u>californicus</u> parcasiticus shorter (0.15-0.40 sec.), low (2.5-20 kHz)

squeaks were found for <u>P</u>. <u>maniculatus</u> <u>gambeli</u>; while the highest squeaks (to 24 kHz) and shortest (0.08-0.17 sec.) were found for <u>P</u>. <u>crinitus</u> <u>stephensi</u> and <u>P</u>. <u>eremicus</u> <u>eremicus</u>.

Hart and King (1966) compared <u>P</u>. <u>maniculatus</u> <u>bairdi</u> and <u>P</u>. <u>maniculatus gracilis</u> and found significant differences in the characteristics of their ultrasounds. <u>P.m. gracilis</u> pups produced more pulses per set, at a higher rate, with a longer interpulse interval than <u>P.m.</u> <u>bairdi</u>, but the squeaks of the latter had a higher maximum frequency and a longer pulse duration. Despite the lower maximum frequency, <u>P.m.gracilis</u> had a wider spread in the dominant frequency (3.6 - 26.5 kHz) than <u>P.m.bairdi</u> (4.9 - 23.7 kHz).

Brooks and Banks (1973) compared the sounds of 2 genera of lemmings, and Noirot (1966a,b; 1968), Okon (1970a, b; 1971), Sales (1972), and Sewell (1968; 1970) have compared sounds across several genera of mice, rats and hamsters. Nitschke, Bell and Zachman (1971) compared 3 strains of albino laboratory mice, C57BL/6J, BALB/cJ, and C3H/HeJ, in terms of rate of signaling, peak frequency, and signal duration. Strains and ages were found to be significantly different on all parameters. Rate measures were apparently made from pooled totals of 3 - 10 second periods separated by 1 minute intervals, a measure not directly comparable to those in any other studies. "Peak



frequency" was unfortunately not defined and it was not clear whether the signals measured occurred in sets. Comparisons with other species are thus difficult. The Hart and King study on the subspecies of <u>Peromyscus</u> (1966) is therefore the only useful study of sound differences within a natural species.

Ontogenetical Changes:

Noirot (1966b, 1972) found that <u>Mus</u> pups showed a change in squeak rate with age. The rate increased until the date of ear-opening (day 4) and then decreased almost to zero on the day of eye-opening. The total number of squeaks also peaked on day 4 and the percentages of sounds of short, constant frequency changed relative to the percentages of very, high squeaks of long, steady frequency. This now appears to be similar to the relative changes Okon (1970a,b) found between "handled" and "cold-stress" stimulated calls. In 1968 Noirot found similar results with white rats. Curiously the call rate appeared to be lowest when the rate of retrieving was highest, that is at about day 4.

Noirot and Pye (1969) found 3 age divisions for the characteristics of the sounds. Over age a slight initial rise in frequency became apparent. By day 4 calls showed less variation and the calls produced on day 12 were few in number but showed a significant rise in frequency. The length of the squeaks decreased with age after day 4 and the

bandwidth and sound pressure level also dropped. Frequencies were found to be more constant on day 4 than earlier or later.

Brooks and Banks (1973) also found 3 age divisions in the sounds of young lemmings. Younger pups, days 3-5, gave simple calls, very short (0.08 sec.) and high frequency (to 29.1 kHz). Older pups had more complex calls with 2 to 3 distinct segments, longer (0.61 sec.) and lower (26.6 kHz) than earlier. Still older pups produced longer squeaks (0.70 sec.) with lower frequencies (19.9 kHz). Under cold stress these authors found the rate of calling by lemming pups to be highest on days 3-4 while rates of calling to pain (tail pinch) were highest from 4-7 days. Other types of tactile stimuli, touching, rolling between the fingers, elicited audible responses before day 2 and ultrasonic responses reaching a peak on day 3. The patterns of change in production of these calls resemble Okon's results (1970a,b; 1971).

In <u>Peromyscus</u>, Hart and King (1966) found a significant age trend with a decrease in rate, a decrease in maximum frequency (<u>P.m.bairdi</u>) or a rise and then a drop in frequency (<u>P.m.gracilis</u>), and changes in pulse duration and interpulse interval.

Okon (1970a,b, 1971) found 3 developmental periods with respect to rate of calling and intensity of ultrasounds. In the early period, where the pups are essentially

poikilothermic, up to 5-6 days, only few, weak responses are made. After day 6 the intensity of cries correlates closely with age, during cold stress, and squeaks are actively produced. Okon found this period to be transitional for thermoregulation where some control is possible. During the last stage, after 13-14 days the intensity of calls depend directly on temperature. Rate of calling declines steadily from an early high.

Smith (1972) describes corresponding stages in <u>Peromyscus</u>, with "audible" pulses at all times approximately 30 db greater than the "ultrasonic" pulses. For the "audible" pulses no changes were observed in number of pulses per set and "ultrasonic" pulses were not produced in any temporal pattern. "Audible" pulses reached a maximum rate on day 7 whereas "ultrasonic" pulses were produced by pups older than 4 days and reached a peak rate on day 10. In this study recordings were only made on days 1,4,7,10,12,14,17 so gradual trends were not apparent.

The consensus of the data discussed above must be that rodent pups do produce ultrasonic (20 kHz and up) and audible (below 20 kHz) squeaks under the stimulus of cold stress and handling. The signal may differ between taxa as small as subspecies and certainly changes in characteristics occur with age. Rate and intensity changes appear to correlate closely with thermoregulatory abilities while other changes may have other causes.

Relevant Receiver - Hearing:

On the other end of the communication system the receiver must be able to hear the sound signal and then must act on the transmitted message for the information transfer to be complete.

Reactions to Preyer's ear muscle reflex and dorsal skin twitches in response to sounds of Galton whistles and sound generators, indicate sound perception at frequencies up to 100 kHz in various rodents (Tembrock, 1968; Davis, 1927). With these reflexes Schleidt (1948) found responses to between 25 and 30 kHz in the bank vole <u>(Cleithrionomys)</u>. Preyer's ear muscle reflex is a measure of perception which may, at worst, overestimate the threshold (Denes, 1961).

Audiograms published as a result of continued experimentation (Schleidt, 1951, 1952) show sound perception in hamsters and mice acute up to 20-30 kHz and extending to about 75 kHz. Dice and Barto (1952) found good sound perception in <u>Peromyscus</u> up to 95 kHz. These authors also found differences in responsiveness between species, with <u>P. nasutus</u> responding to frequencies as high as 100 kHz. These differences were believed to be correlated with the different ear sizes of the mice.

Electronic recording from implanted electrodes provided Ralls (1967) with more precise measurements of sound perception in <u>Peromyscus leucopus</u>, <u>P. boylei</u>, and <u>Mus</u> <u>musculus</u>. The <u>Peromyscus</u> mice were found to have greatest sensitivity to high frequencies up to about 40 kHz and some

sensitivity up to 70 kHz. Younger animals showed better responses between 30-40 kHz than 6-month old animals. The albino mice showed a low threshold between 5-30 kHz, with best response at about 15 kHz. These data agree with the results of Berlin (1963), who found albino mice most sensitive at 15 kHz, with a range of sensitivity from 1-70 kHz, using the galvanic skin response.

Thus it seems clear that deermice, and other rodents, can hear the ultrasonic sounds produced by their stressed young, and that in the lab at least, these sounds could serve as stimuli in maternal retrieving.

Responses - Maternal Retrieving Behavior:

That the particular activities, such as nest-building, retrieving, nursing, and cleaning, commonly grouped as "maternal" behavior must be considered separately in any discussion of cause and function has been amply demonstrated by Beniest-Noirot (1958), Lazar (1967), Richards (1967) and Slotnick (1967). Although each of these activities is epimiletic, care-giving (Scott, 1958), in regard to the young of the species, they occur independently and each appears to be related to different combinations of casual factors (Richards, 1967). In most cases these causal factors can be grouped into three classes; the stimuli from the pups (extrinsic), the previous experience of the mother (both extrinsic and intrinsic), and the hormonal condition of the mother (Richards, 1967).

In a review of maternal behavior in rodents and lagomorphs, published in 1967, Richards discusses the role of each of these classes on the several, separate maternal behaviors. In the present discussion I will review the data with respect to retrieving behavior and summarize the additional papers published since 1967.

General Parameters:

King (1963) cites field observations of <u>P</u>. <u>leucopus</u> and <u>P</u>. <u>maniculatus</u> mothers returning to young scattered during nest disturbances and carrying them to new refuges. In a laboratory study, <u>P.m. gracilis</u> and <u>P.m. bairdi</u> did not show significant differences in retrieval latencies, although there was considerable variation in the behavior of the subjects while retrieving (King, 1963). King also noted that the nature of the interactions between the mother and the pups changed as the pups grew, until no pups were retrieved at 30 days of age.

With mice, rats, and occasionally with hamsters, the mere presence of live pups near a nest is sufficient to evoke retrieval responses from males as well as virgin, pregnant, or lactating females (Beniest-Noirot, 1958; Richards, 1966b, 1967). Richards (1966a) and Rowell (1960) found that virgin female hamsters killed and ate pups up to the age of 5 days, but often retrieved older pups. Noirot (1964) found that with naive female lab mice, younger pups, 1 and 2 days old were most effective at

eliciting retrieving and other maternal behaviors. As the pups grew their effectiveness decreased. Both Noirot and Richards suggest that the change noticed in the retrieving behavior of the females is related to the change in the stimulus value of the pups - possibly the distress calls (Noirot, 1964b; Richards, 1967). The relationship of retrieving to the presence of ultrasonic distress calls is also indicated by the fact that the incidence of retrieving drops off at the age at which the young cease emitting the calls (Noirot, 1966a,b; Zippelius & Schleidt, 1956).

Priming:

Early contact with the strong stimulus of a live oneday old pup can cause a higher percentage of both male and female mice to respond to a "weak" stimulus (a day-old drowned pup) even if it is presented as much as 8 days later (Noirot, 1964a). Similar results were later found with hamsters (Noirot & Richards, 1966). This increase in response probably depends only on exposure to "one or more essential cues provided by the [pup]" (Noirot, 1964a).

When naive female mice were exposed to a live, but hidden pup, their maternal responses, including retrieving were greatly increased when subsequently tested with a dead pup (Noirot, 1969a). Since there was no common cues, sound and odor lacking with the dead pup, this increase in responsiveness is more a "sensitization" or "priming." The priming of maternal behaviors by initial contact with a

strong stimulus is selective. Olfactory cues appear to increase subsequent pup cleaning, while auditory cues favor nest-building (Noirot, 1970). Retrieving may also be primed by auditory cues, but Noirot (1970) hypothesized that this experimental situation elicited both distress and contact (handled) calls from the stimulus pups and may have complicated the situation by opposing effects of retrieving behavior.

Sensory Parameters:

Beach and Jaynes (1956c) deprived retrieving mother rats of vision, olfaction, and sensation around the snout and found that lack of reception from any one of these senses reduced their efficiency at retrieving. Earlier, Weisner and Sheard (1933) found similar results in an experiment where they also blinded the rats. Loss of any two of these senses produced a greater effect than the lack of only one. However, Beach and Jaynes (1956o;c) did not consider audition and they regarded retrieving as a whole behavior sequence from the first excursion from the next until the return. Each different sensory modality might be primarily effective at a particular part of the retrieving pattern. In another paper, the same authors do conclude that olfaction is an important sense in the discrimination of known and unknown young (1956a).

The papers discussed above lend support to the theory that some part of the stimulus configuration supplied by the

helpless pup affects the retrieving mother's response. Evidence for the existence and importance of ultrasonic sounds as a primary cue has already been discussed.

Effects of Experience:

The hormonal and experiential state of the mother, intrinsic factors, have considerable relevance to her readiness to retrieve. Richards (1967) outlined several categories of previous experience which might affect the retrieving behavior of a rodent, including in this list: 1) the effects of manipulations in infancy; 2) the effects of manipulations during pregnancy; 3) the effects of parity and; 4) the effects of practice. Other experimental factors mentioned relate more to other epimiletic behaviors than to retrieving <u>per se</u>, and so are not included in this list.

The manipulations of the mother as a pup, or her mother affect the behavior of the mother towards her pups (Levine, 1967; Meier & Schutzman, 1968; Richards, 1967). Oppositely, Young (1965) demonstrated that the treatments of the young (rotation or cooling) influenced the mother's behavior towards them. This may be particularly important if indeed the calls of the young relate to the aggressive behavior of the mother to them. In the Young study, non-treated pups were retrieved more quickly than treated littermates. Meier and Schutzman (1968) in a review of the data on the effects of experiemtnal treatments on mother-infant interactions, stress the complexity of the interaction. Any experimental intervention



involving the young also introduces changes in the epimiletic behavior of the mother (Meier & Schutzman, 1968). It is the mother's changed behavior which then influences the development of the young.

Further evidences of the dynamic interaction between mother and infant are given by cross-fostering studies done on lab mice and described by Meier and Schutzman (1968; Ressler, 1962). In this case survival of fostered young was found to be better with BALB/c mothers than with C57BL/10 mothers, whether the strain of the pups was BALB/c or C57BL/10. The authors conclude that the behavior of the mother is "shaped" by the responses of the pups and vice versa.

Any manipulations which alter the emotionality of the mother as a pup, including her own mother's behavior, may well affect her behavior towards her own pups. Unfortunately few studies of these effects on rats and mice measure differential effects on different "maternal" behaviors such as retrieving.

A less well explored area is the effect of manipulations during pregnancy. Studies of the effects of total sound restriction during pregnancy (Levine & King, 1965) and exposure to anxiety provoking conditions during pregnancy (Richards, 1967) demonstrate reduced offspring survival and reduced open field activity. Levine and King (1965) suggest that the observed effects may be due to a general disorganization of maternal behavior.

Restriction of self-licking during pregnancy, may cause total failure of maternal behaviors and this may either be due to the failure of the development of a continuity between the mother's own body and those of the young or to the underdevelopment of the mammary glands due to lack of stimulation (Richards, 1967). At the time of parturition normal rats switch extensive licking from their own bodies to their young. Other maternal behaviors increase in rate of emission at this time also, but the patterns of the switch are not clear.

Experiences with pups have more direct effects of the development of maternal behaviors. While even primaparous females make good mothers the consensus of published work is that subtle behavioral tests can detect improvements in style during the raising of any one litter and from a first to subsequent litters (Beach & Jaynes, 1956b; Carlier & Noirot, 1965; Richards, 1967). Disagreement as to the carryover of improvements from one litter to the next indicates that the interactions are subtle.

With respect to retrieving in rats, Carlier and Noirot (1965) demonstrate a reduction in time to retrieve in biparous rats over their earlier litters. This improvement may be due to increased skill in transporting the pups, the biparous mothers carry the pups more efficiently by the central venter rather than elsewhere (Beach & Jaynes, 1956b; Carlier & Noirot, 1965; Richards, 1967). Additionally, decreases in time to retrieve may be due to priming, as evidenced by a

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decrease in the time spent in the nest by retrieving mice & ground squirrels (King, 1958; Michener, 1971).

Recognition of the stimuli emanating from the pups may also play a part in changes due to experience. Given enough time with pups of their own species, male rodents of several species and virgins will retrieve and attempt to care for the pups (Beniest-Noirot, 1958; Gandelman, Zarrow & Dennenberg, 1970; Richards, 1967; Rosenblatt, 1967, 1970). Even in natural situations male rodents may care for pups (Horner, 1947; Horner & Taylo-, 1969). Whether housed in groups where males and virgins constantly were in contact with pups (Gandelman, Paschke, Zarrow & Denenberg, 1970) or individually offered pups of various ages for several daily tests (Beniest-Noirot, 1958; Richards, 1967; Rosenblatt, 1967) generally only slight differences in latency differentiated these groups from parous females. In a single-trial test situation, however, male P. polionotus seemed more critical of the species of test pups than parous females. The males usually attacked test pups (P. maniculatus) but the parous females never did. In this test both males and females had lived with litters of pups (experienced). Inexperienced males and females tended to bite both con-specific and non-specific pups (S. Huff, unpublished observations).

Although it is apparent that a level of response to young exists in most adult rodents, even with previously paroud females the effects of priming are noticeable. With Richardson's ground squirrel Michener (1973) found that



more non-lactating females retrieved than virgins. This suggested that with this animal the prior experience of raising a litter has a lasting effect. In particular the results of such tests depend on the quality of the stimuli. Very young pups seem to be stronger stimuli than older ones. Grota (1973) found that with new-born pups as stimuli the maternal behavior of rats is related only to the age and the size of the litter while the previous experience of the mother is more important with older pups. Noirot (1964a,b,&c, 1965; Richards, 1967) suggested that contact with a very young pup, in mice and hamsters can increase responsiveness to older All of these studies and the discussion in Richards pups. (1967) lead to the conclusion that the interaction between mother and pup in a normal situation involves both practice in performance of the maternal behavior by the mother and recognition of the stimuli produced by the pup. Both are constantly being revised by the mother as a result of feedback from previous contacts with the pups. The stimuli emanating from the pups may also change in response to the mother's actions; i.e., a pup may squeak more if not retrieved or handled roughly.

Hormonal Effects:

The hormonal state of the mother relates directly to her readiness to perform any of these behaviors. Where retrieving was made difficult (leaving home cage) Gandelman, <u>et al</u> (1970) found lactating female mice more willing to

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retrieve than virgins.

Much discussion in the literature has centered on the role hormones play in the induction of maternal behavior. The role of hormones in the development and maintenance of the pregnancy which provides the pups is undisputed. However, Rosenblatt (1970) states that "there is no clear evidence that the mother requires either ovarian or pituitary hormones for the maintenance of maternal behavior after parturition." Differences between the gradual development of maternal behavior in virgins and newly parturient females may be as much due to the physical events of parturition as to the presence of specific hormones. While the induction of maternal behaviors at parturition may be related to hormones its maintenance appears to be almost completely due to stimuli from the pups (Rosenblatt, 1970).

Confusion between the effects of hormones on maternal behavior and pup stimulation has often arisen from failure of authors to differentiate various maternal behaviors. For example, Leon, Numan and Moltz (1973) conclude that maternal behavior is normally under inhibitory gonadal control, as demonstrated by increased levels of maternal behavior as long as 8 weeks after gonadectomy, in both males and females. The undoubtedly subtle effect of decreases in estrogen is obscured by the failure to either differentiate the hormones or behaviors. Similarly Thoman and Levine (1970) demonstrate that a functioning adrenal is not necessary for maternal behavior in rats. Of course, hormones, prolactin particularly,



contribute to the maintenance of lactation and thus have an indirect role in the continuance of maternal behavior (Richards, 1967). A study by Moltz (1970) found that ovariectomy disturbs maternal behavior in primaparous rats but not multiparous, presumably experiential factors are superimposed on the hormonal state here.

Much of the disagreement may be due to the different roles hormonal preparation plays in the onset of different maternal behaviors (Richards, 1967). While next-building is due to the reversal of the progesterone/estrogen (Lisk, Pretlow & Friedman, 1969; Lisk, 1971; Richards, 1967) retrieving may be due entirely to stimuli from the pups.

Epimiletic behavio- itself does not change the hormonal constitution of the animal displaying it, at least in rodents. When Terkel and Rosenblatt (1971) cross-transfused blood from retrieving virgins to other virgins no effects of the blood alone was observed on the retrieving behavior of the recipients. The latencies to retrieve were found to be directly proportional to the size of the cage and the closeness of contact with the pups! If an injection of maternal blood plasma or cross-transfusion is done with mothers within 30 minutes after parturition an effect is found on lowering the latency to retrieve (Rosenblatt, 1970; Terkel & Rosenblatt, 1968).

In summary it now appears clear that while some "maternal" behaviors are hormonally primed at parturition, particularly by the changing ratios of estrogen and progester-

one, only nursing and possibly, next-building require the continued presence of hormones (List, 1971; List <u>et al</u>., 1969; Rosenblatt, 1970; Terkel and Rosenblatt, 1971). Even these behaviors and retrieving and cleaning require continuous stimuli from the pups.

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Stimuli Emanating from Infants:

The entire <u>Gestalt</u> of the neonatal rodent pup may play a stimulus role in the elicitation and maintenance of the care-giving behavior of adults. Specific elements of this <u>Gestalt</u> are difficult to extract and study separately. Nevertheless, Noirot (1970) demonstrated that olfactory cues tend to prime cleaning behavior while auditory cues prime nestbuilding and retrieving. Many earlier studies suggest that auditory cues are important guides in retrieving behavior and may also serve to inhibit adult aggression (Hart & King, 1966; Munn, 1950, Noirot, 1966; Zippelius & Schleidt, 1956). Sound seems to stimulate retrieving; silent pups are often not retrieved (Gandelman, <u>et al</u>., 1970; Noirot, 1964a; Sewell, 1970; Wiesner & Sheard, 1933).

Since the stimulus value of the pup changes with age (Noirot, 1964a,b,c, 1966) and the mother's readiness to retrieve also changes (King, 1958), the separate elements of the pup-stimulus must change. The odor of the pup may be dependent on its food and the odor of its family and thus not likely to change until weaning and dispersal. The pups are constantly growing and growing hair, changing the visual



picture, but these changes have not been correlated with any alteration of the behavior of the mother.

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However changes in the sounds and ultrasounds (above the range of human hearing, about 20kHz) are readily measurable and appear to correlate with maternal readiness to retrieve. As silent pups are not retrieved, retrieving drops off about the time of the development of adult temperature regulation, which corresponds to the time of eye opening (Beniest-Noirot, 1958; King, 1958; Noirot, 1972; Richards, 1967; Zippelius and Schleidt, 1956).

Ecological Considerations:

Nicholson (1948) relates observations on movements of young litters by wild female <u>Peromyscus</u>. Flooding of nests or disturbance by predators may be included among factors which would prompt a female to move her litter. It is also probable that some nest shifting occurs spontaneously during the nurturance of any one litter (Eisenberg, 1968; King, 1963). When disturbed, feamle <u>Peromyscus</u>, <u>Apodemus</u>, and other mice flee the nest with their pups attached to their nipples (King, 1963; Sewell, 1968; Huff, pers. obs.). The pups cling so strongly that the mother can be lifted by picking up a pup. The mother herself may have difficulty removing pups, although usually she disengages them before leaving the nest on routine errands (King, 1963, 1968). The strong attachment of the pups provides an efficient means of transporting several pups simultaneously in a hurry, but weaker pups do fall off and are


left behind, while the mother continues to a new refuge (1968, Layne, King, 1963). Once in the new nest the female disengages the remaining pups and returns to retrieve squeaking pups left behind. These observations are consistent with the theory that the ultrasonic squeaks function to keep the pups with their parent.

Localization:

Further confirmation of the importance of the sounds for this behavior is found in Allin and Banks, (1972). In this study male white rats, virgin females, and lactating females were placed in the center of a hexagonal platform and tested with recorded distress cries from a 7-day old pup. Fifty percent of the lactating females made at least one sortie out of the central nest box towards the peripheral sound source. Males and virgin females did not sortie but did orient to the sounds. These groups also reacted to the control noise. No appreciable differences were found between the recorded sounds the rats heard and the sounds originally recorded, so the authors concluded that the sounds contained sufficient clues for accurate orientation to the source without odor or sight of the pup.

Discrimination:

Adult <u>Peromyscus</u> show preferences for mates of their own species on the basis of odor (Blair, 1953, 1954; Blair & Howard, 1944; Bradshaw, 1965; McCarley, 1964; Moore, 1965).



It should be possible for adults to discriminate pups by odor. Adults will reject foreign pups even of their own species and often of other species and this is commonly assumed to be due to a "strange" odor. Whether sound plays a part in discrimination has not been demonstrated except as will be discussed in this paper.

Predators:

If the sound or the excursion to retrieve a lost pup created a ready target for predators it would be hard to see how the behaviors could be maintained. That other mammals, including predators, can hear such sounds is known. Peterson, Herton, and Wruble (1969) measured cochlear microphonics in fissiped carnivores at the second tympanic membrane. At this level those small carnivores which might be mouse predators, such as coyotes, dogs, foxes, raccoons, and cats, demonstrated sensitive hearing in the regions of the frequencies of mouse squeaks, with upper limits at 60-100 kHz. Conversely, Payne (1962) found that barn owls appear to be guite deaf to sounds above 15-20 kHz. Instead, they are capable of accurately locating mice by the sound of their movements in leaves. Predators could thus either hear the squeaks of lost pups, or the sounds of mothers moving to retrieve them and the situation must contain substantial risk. The efficiency of the mother's localization of the sound must be great enough for it to be ecologically more efficient to retrieve, than to lose



the genetic and energetic investment on the pup.

Summary:

Data available in 1968, when this study was undertaken, and data published since, agree that infant rodents produce ultrasounds to cold-stress and handling. These squeaks, when heard by mothers, trigger maternal retrieving behavior. Information on the age of the pup and possible species may be included in the sound. These sounds were seen to have complex harmonics and frequency changes. The utility of the sounds to a wild mouse mother is suggested. The study to be described in the next chapters analyzes distress calls of several species in one genus, compares them in detail across age and species and shows their relevance to the listening mouse.



CHAPTER 3

Species-Specific and Ontogenetic Characteristics of the Peromyscus "Distress" Cry.

Introduction:

Noirot (1966b) found qualitative and quantitative changes in ultrasounds emitted by albino mouse pups as they matured. Hart and King (1966) found changes in squeak frequency with age in <u>Peromyscus maniculatus</u> pups and a decrease in numbers of sets of squeaks per 30 seconds with age in the same mice. Frequency changes may be associated with maturation of the vocal tract (Hart & King, 1966) or the opening of the ears to permit auditory feedback. Large changes in frequency and emission rate are positively correlated with the opening of the ears (Noirot, 1966b). Decreases in squeak rate or frequency are correlated with the onset of thermoregulation and the development of fur (Hart & King, 1966; Okon, 1970a).

The various behavior patterns defined as maternal by Beniest-Noirot (1958), change in intensity and threshold of action in mother hamsters, deermice, and house mice as their litters age (King, 1963; Noirot, 1966a; Richards,

1966a,b; Rowell, 1960). This may be partly due to changes in hormone levels (Noirot, 1969a; Terkel and Rosenblatt, 1968) or partly or wholly due to changes in the stimulus characteristics of the pups (Noirot, 1964b; 1966a; Richards, 1966a,b; Rosenblatt, 1967). Visual and auditory characteristics change more than olfactory characters during the neonatal period but although gradual changes due to growth and development of the body and pelage are visible to human observers they do not correlate with the sharp decline in maternal responsiveness which occurs at about the time the pups open their eves. Mouse pups stop emitting ultrasonic distress calls at this time, however, and the cessation of squeaking correlates with the decline in maternal behavior (Noirot, 1964b; 1966a). Moreover, Noirot (1970) has demonstrated that ultrasounds selectively prime and maintain retrieving and nest-building while other stimuli (olfactory) prime other aspects of maternal behavior such as cleaning.

In the analysis reported here I concentrated on identifying the quantifiable parameters of <u>Peromyscus</u> distress squeaks and describing the ontogenetical changes through which they go. Certain aspects of these sounds may carry particular sorts of information and may change in different directions or at different rates. Changes in frequency might be expected to correlate best with the development of sound production and monotoring organs while rate of emissions might correlate with thermo-



regulatory abilities. Characteristics, such as redundancy, frequency and intensity modulation and sound envelope shape, which aid adults in locating squeaking pups, are predicted to change only in the direction of increased effectiveness until the pups no longer need to be retrieved.

The sounds produced by many animals are used for species identification by the animals themselves and by human observers. Species-constant sound characteristics were therefore expected to occur in the <u>Peromyscus</u> squeaks both as incidental results of differential body shapes and as characters selectively divergent under pressures of sympatry. The subspecies of <u>P.maniculatus</u> should have the most closely related frequencies if vocal tract muscles change more slowly than behavior but these same mice should have more divergent squeak rates, durations, and modulations where they occur in sympatry. If the sounds could be analyzed precisely enough it should even be possible to suggest taxonomic relationships on the basis of differences and similarities between the calls.

A refinement towards some species-typical pattern in the sound would be expected if these sounds are used by the mice for identification. Additionally, increased ability to thermoregulate as the pups grow would reduce the motivation to squeak from cold stress and increasing mobility would make it less important that the pup have its mother come to retrieve it. Both these changes should be expressed in shorter squeaks, lower squeak rates and fewer



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squeaks emitted.

Procedure:

Subjects:

Systematic recordings were made of Peromyscus pups from day one after birth to eye-opening. The day of eyeopening was considered to be the average date for each species as determined from colony records and preliminary studies (Table 3-1). Pups with both eyes open did not squeak and those with only one open rarely did, so that most of the pups recorded did not have open eyes. Daily recordings were made of six separate individuals from four taxa, P.maniculatus bairdi, P.m.gracilis, P.polionotus, and P.leucopus. Recordings were also made of pups from P.californicus, P.difficilis, P.melanophrys, and P.m.nebracensis when they were available. Due, however, to small numbers of these taxa in the colony and the infrequency of litters it was not possible to record as many individuals at as many ages as the first group. Pups of P.californicus were recorded on days 1, 2, 4, 6, 8, 10 (12 individuals), P.difficilis on days 8, 10, 12, 14 (6 individuals), P.melanophrys on days 2, and 4, and P.m. nebracensis on day 4 (2 individuals). The females of these species often cannibalized an entire litter after one pup had been recorded, thus reducing the supply of pups.

No more than four mice were used from the same litter and no mouse was used twice. If there were more than



Table 3-1

Ages of eye opening and ear opening in species of <u>Peromysous</u> in the Michigan State Colony. I bata fram colony records and unpublished study by J. Huff and B. Vestal.

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Species	ч	age_eye opening x days SE	age_ear opening x days SE	d <u>i</u> fference x days	д	
P.m.bairdi	32	12.9 ± 0.1	11.7 ± 0.2	1.2	.001	í.
P.m.gracilis	15	14.7 ± 0.5	13.3 ± 0.3	1.7	.05	
P. leucopus	51	12.4 ± 0.1	11.7 ± 0.1	0.6	.001	49
P. polionotus	31	13.7 ± 0.3	12.5 ± 0.4	1.1	.01	
P. californicus	2	14.2 ± 0.4	13.6 ± 0.7	1.7	n.s.	
P. difficilis	6	17.4 ± 0.2	16.3 ± 0.2	1.1	n.s.	
P.melanophrys	2	14.0	14.5	0.5	n.s.	
P.m. nebracencis	44	13.1 ± 0.09 (King,	, 1970)			

A Statement



two pups in a litter, a toe was clipped on each recorded individual so that it could be recognized and avoided on subsequent days. No more than two pups from any litter were recorded on the same day, in order to avoid possible family influences.

Care and Handling:

All of the mice were maintained in the mouse colony room in clear, plastic cages (5 x ll x 6 in deep) with removable wire lids holding food and water bottles. Lighting was kept at a summer cycle, 12-14 hours light; 12-10 hours dark. Unless there was a history of litter cannibalism males were left with the females and their litters at all times. Pregnant females were checked twice daily for litters, although for the purposes of this study ages were counted by 24 hour periods.

At the time of recording, pups were removed from the maternal nest in pairs, if possible, and carried into the recording chamber in the experimenter's hands to reduce chilling. The pup to be recorded first was placed in the apparatus while the other was held in a box under a desk lamp a sufficient distance from the microphone.

Recording Procedure:

Before squeak recording the mouse pups were examined for open eyelids and then placed on a wire square above a cotton plug in the bottom of a large metal funnel (Figure

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3-1). This apparatus restricted the movements of the more active pups and kept them within an average range of the microphone. The close contact of the metal walls may have also stimulated them to squeak more (Noirot, 1970). The microphone was then lowered to within three inches of the mouse, the experimenter left the room, and the sound monitoring system was turned on.

If the mouse did not squeak in five minutes it was returned to the nest and another pup was used. When the pup appeared to be squeaking consistently the tape recorder was turned on and sounds were recorded for at least one minute. If the mouse stopped squeaking for more than 15 seconds the tape was stopped until the mouse resumed squeaking. The recorded sound was constantly monitored with an oscilloscope and headphones.

After recording the squeaks the mouse was removed from the funnel and checked for raised pinnae and a startle response to a loud click, then returned to its nest.

Recording Equipment:

Sounds were picked up from the squeaking pups with a 1/2 inch Bruël and Kjaer condenser microphone, amplified by a Bruël and Kjaer microphone power supply and Tektronix amplifier (Appendix A). These sounds were recorded with an Ampex four-channel instrumentation recorder on Ampex instrumentation tape (1 mil mylar) running at 15 ips. A playback head on the recorder allowed the sounds to be



Figure 3-1 Legend

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Mouse restraining chamber for squeak recordings. The mouse pup (m) was placed at the bottom of the funnel (F) and the microphone (M) was lowered to within 3 inches of the pup. A ring stand (R) and clamps and a large can (C) supported the microphone and funnel. (MC) is the microphone cable.



Mouse Recording Chamber



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simultaneously displayed on a Tektronix oscilloscope. The tape recorder was the limiting factor in this system, in terms of frequency response, as it was rated as giving a flat response to sounds only from 4 to 40 kHz at 15 ips. The microphone gave a flat response to 50 kHz and acceptable sound recording was obtained to 60 kHz. Sounds above those frequencies were observed in the recordings, but relative sound energy levels could not be compared. (For more detailed equipment specifications see Appendix Al).

Although the recording chamber was sound-damped and all the available 60-cycle filters were used, on some days loose connections or high background noise made the recordings too noisy to be analysed. When a recording of a particular age was critical, more than six mice were recorded at that age to ensure a sample of clean records.

Sound Analysis Methods:

All of the one-minute samples of squeaks for each age of each species were filmed at normal tape speed (15 ips) using a Tektronix oscilloscope and a Grass Oscilloscope camera. All of them were filmed with a time base of 1 cm per second and some of the clearest were also filmed at 2.5, 5, and 10 cm per second. The resulting oscillograms showed the temporal relationships of the squeaks and permitted measurements to be made of the duration (in seconds) of individual squeaks, groups of squeaks (sets), and inter-squeak intervals, and inter-set intervals, as



well as permitting the numbers of squeaks per set to be counted.

Most of these clear recordings were also analysed with a Bruël and Kjaër Sound Frequency Analyser which graphed a spectrum of sound energy, from a single squeak, in terms of sound pressure level (db) present in particular frequency bands. In order to register, the tapes had to be slowed to 1-7/8 ips. These spectrograms showed relative sound energy and harmonics and frequency modulations more precisely than the other methods of analysis. Because the mice were free to move somewhat in relation to the microphone and because they changed their sound volume at will it was impossible to calculate absolute sound levels from the spectrograms. However, relative sound pressure levels could be compared between parts of any one squeak. The individual excursions of the pen drawing the spectrograms could also be compared to the sound heard from the squeak being analysed and thus frequency modulations within the squeak could be recognized.

Those squeaks spectrographed were also examined with a Voiceprint machine which made sonagrams of the sounds. Again the tapes were slowed to 1-7/8 ips. Sonagrams are a standard means of visually presenting bird songs and human speech but, although they are used increasingly for non-human mammal sounds, the quantifiable data they provide from these simpler calls is meager. Pictorially a sonagram furnished an attractive comparison

between sounds and for this reason a number were done in this study. Sonagrams show frequency over time, and intensity can be estimated from relative darkness of the burn. Since the sonagrams depict a mouse squeak spread out over time they were compared to the sound spectrograms which showed precise frequencies and the oscillograms which showed time and intensity.

Parametric or non-parametric analyses of variance were done on the temporal data, in detail for those taxa with samples from more than 12 individuals (Group A, <u>P.m. bairdi</u>, <u>P.m.</u> <u>gracilis</u>, <u>P. polionotus</u>, <u>P. leucopus</u>) and more superficially with the smaller samples from other taxa (Group B, <u>P.</u> <u>californicus</u>, <u>P.m. nebracensis</u>, <u>P. difficilis</u>, <u>P. melanophrys</u>).

Sound frequencies were calculated from the sound spectrograms and compared with the sonagrams. Mean basic frequencies*, fundamental frequencies**, and means for major harmonics were calculated where possible for ages and taxa. When fundamental frequencies were present the other frequencies were compared to the fundamental to determine where they fit into the overtone series. If the fundamental was absent an attempt was made to estimate it.

Differences in intensity modulation, frequency modulation, and changes in pitch were visible in the sound sprectro-

^{*}The most important (largest amplitude) frequency component.

^{**}The frequency of repetition of the complex wave (Ladefoged, 1962).



grams and like the temporal changes visible in the oscillograms could be objectively compared from the pictures. These differences are displayed in the plates included in the results section. The sections of films shown in these plates were chosen for typical patterns as well as clarity and low signal-to-noise ratios (Figures 3-2 to 3-14).

Results:

Species Differences - General Observations:

The null hypothesis was that squeaks of different species or subspecies would not be significantly different. Data analyses disproved this and showed differences on all measured parameters. Squeaks of 8-day old pups were observed to be clear and relatively invariant within a taxon when they were compared across all the taxa, characteristic patterns became apparent (Figures 3-2 to 3-8). Oscillograms and sonagrams showed that P.m.bairdi, P.leucopus and P.californicus start each squeak with a rapid frequency and intensity rise, modulate both and then suddenly drop off (Figures 3-2,3). Other taxa, P.m.gracilis, P.m.nebracensis, P.polionotus, P.difficilis, show an initially sharp frequency rise followed by a more gradual one to a peak and then a decrease (Figure 3-2). P.m.nebracensis, P.m.gracilis, and P.difficilis particularly appear to put the greatest emphasis on the middle squeak in a set (Figures 3-2,3).

While intensity modulations are most visible on the oscillograms, frequency variations are clearer on the



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Figure 3-2 Legend

Oscillograms of distress cries from 8-day old <u>Peromyscus</u> pups. (a) <u>P.maniculatus bairdi</u>; (b) <u>P.m.gra-</u> <u>cilis</u>; (c) <u>P.m.nebracensis</u>; (d) <u>P.leucopus</u>; (e) <u>P.polio-</u> <u>notus</u>; (f) <u>P.californicus</u>; (g) <u>P.difficilis</u>. Time marks are 1 cm equal to 1 second. Similar intensity modulation patterns are visible in the squeaks of <u>P.m.bairdi</u>, <u>P.leu-</u> <u>copus</u> and <u>P.californicus</u> with intensity decreasing in the center of the squeak. <u>P.m.nebracensis</u>, <u>P.m.gracilis</u>, <u>P.</u> <u>polionotus</u>, and <u>P.difficilis</u> exhibit greatest intensity in the center of the squeak and often also in the central squeak of a set.





Figure 3-3 Legend

Oscillograms compared with sonagrams. <u>P.leucopus</u>: (a) an oscilloscope tracing of squeaks from 8-day old pup; time on the abcissa, 5 cm = 1 second, intensity on the ordinate; (b) sonagram of a single squeak from an 8-day old pup; time on the abcissa, mark = 0.1 second, frequency on ordinate, wide-band, log plot. <u>P.maniculatus gracilis:</u> (a) oscillogram of squeaks from an 8-day old pup, 5 cm = 1 second; (b) sonagram of a single squeak from an 8-day old pup, time mark = 0.1 second. The typical strong overtone is visible on the <u>P.m.gracilis</u> sonagram.





sonagrams and spectrograms. <u>P.leucopus</u> and <u>P.polionotus</u> rarely show harmonics (Figures 3-4, 5, 6); most of the sound energy in their squeaks being concentrated in a single frequency. The <u>P.maniculatus</u> subspecies (Figures 3-3, 4) and <u>P.californicus</u> (Figure 3-6), on the other hand, usually show at least one harmonic band. In the case of <u>P.m.gracilis</u> and <u>P.californicus</u> this harmonic is often as strong or stronger than the lower frequency (Figures 3-5,7).

Sound spectrograms (Figure 3-5) reinforce the interpretation of single clear tones from <u>P.leucopus</u> and <u>P.polio-</u> <u>notus</u> and multiple harmonics from the <u>P.maniculatus</u> mice and <u>P.californicus</u>. In addition, careful study of these records enabled me to differentiate between those mice (<u>P.maniculatus</u>) which show a slight rise in frequency at the beginning of the squeak and those (<u>P.polionotus</u> and <u>P.</u> <u>leucopus</u>) with sharper onsets and offsets. Although I was unable to observe different methods of vocalizing it may be that whether the mouse starts squeaking with its mouth open or closed determines the pattern. A sharp, pure tone would be expected to emanate from an open mouth, while a rising frequency might be produced by opening the mouth after the squeak had already been started.

Some of the squeaks show breaks in the solid pattern of sound (Figure 3-6d). Behavioral observations were unable to clarify the relationship of these breaks to the squeaks but as they also appear in the squeaks of mice recorded elsewhere on other equipment, it would seem that

Figure 3-4 Legend

Sonagrams of single squeaks from recordings of 8-day old pups. Time mark = 0.1 second. (a) <u>P.leucopus;</u> (b) <u>P.polionotus;</u> (c) <u>P.maniculatus bairdi;</u> (d) <u>P.m.</u> <u>gracilis</u>. A strong harmonic is visible in the <u>P.m.bairdi</u> sonagram as well as in the <u>P.m.gracilis</u> which also shows a secondary frequency. Compare with oscillograms in Figure 3-5.








FIGURE 3-4



Figure 3-5 Legend

Comparison of oscillograms and sound spectrum graphs. Time marked on the oscilloscope tracings. Sound spectrograms show a plot of sound pressure level (db) at various frequency bands across the abcissa. Each oscilloscope trace displays a single set of squeaks; each spectrum shows a single squeak from the same or a different individual: (a) and (b) P.leucopus, two, 8-day old females; (c) and (d) P.polionotus, one, 8-day old female; (e) and (f) P.m.bairdi, two, 8-day old males; (g) and (h) P.m.gracilis, one, 8-day old female. In all cases the oscillograms and the spectrograms (d) and (f) are from the same individuals as in Figure 3-4. Visual comparisons of the sonagrams and spectrograms reinforce the interpretation of single clear frequencies for P.leucopus and P.polionotus and multiple harmonics present for P.m.bairdi and P.m.gracilis. Apparent double images in the spectrograms are caused by increasing or decreasing frequencies over time.



FIGURE 3-5



Figure 3-6 Legend

Sonagrams of single distress squeaks. Time mark = 0.1 second. (a) 8-day old <u>P.californicus</u>, unusually short and frequency modulated; (b) 1-day old <u>P.polionotus</u> (c) 8day old <u>P.leucopus</u>, second squeak in a set, similar to Figure 3-4a; (d) 7-day old <u>P.leucopus</u>, showing a buzz-like break.



FIGURE 3-6



Figure 3-7 Legend

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Sonagrams of single squeaks. Time mark = 0.1 second: (a) 8-day old <u>P.californicus</u>; (b) 4-day old <u>P</u>. <u>melanophrys</u>; (c) 8-day cld <u>P.m.nebracensis</u>; (d) 8-day old <u>P.difficilis</u>. Compare with Figure 3-8.



they are not experimental artifacts. Such breaks are most common in the squeaks of species showing the greatest frequency and intensity modulations. They also appear to contain more harmonics than the rest of the squeak and to occur at points of great intensity. It may be that these breaks are tongue clicks.

Species Differences - Frequencies:

The hypothesis that the sounds of these taxa would differ in terms of frequency was confirmed by the observations of different modulation patterns (envelope shape) described above. Preliminary analyses also suggested that more precise frequency measures would show significant differences. Mean basic frequencies, i.e. those frequencies with the greatest sound pressure levels in the squeak, were computed for each taxon and each age for Group A (P.maniculatus bairdi, P.m.gracilis, P.leucopus, P.polionotus). Within this group, mean basic frequencies differed significantly at P < 0.001 (Kruskal-Wallis one-way analysis of variance). Overall mean basic frequencies for Group B (P. californicus, P.difficilis, P.melanophrys, P.m.nebracencis) also differed significantly, 0.05 > P > 0.0.1. (Kruskal-Wallis one-way, analysis of variance). Nevertheless the mean basic frequency, averaged over all ages, did not appear to be representative of the characteristics of the sounds. On closer examination two subtleties in frequency characteristics become apparent. A tendency for some species to

produce a squeak with several strong frequencies will be discussed below and abrupt changes in squeak patterns at critical ages will be discussed under ontogeny.

Frequency Patterns:

Some species (P.m.gracilis, P.difficilis, P.californicus in particular) produce maximal energy at two frequencies: in effect two "basic" frequencies. These frequencies were always harmonically related to each other but appear to be in a separate scale from other visible frequencies. The higher of the two "basic" frequencies, usually 34-38 kHz, was always double the lower, usually 18-19 kHz. I labeled those squeaks showing one strong frequency, Type I squeaks, and those showing two strong frequencies, Type II. When the two types of squeak were separated, the daily mean basic frequencies for P.m.bairdi (22.6 kHz), P.leucopus (27.4 kHz), and P.polionotus (33.1 kHz) were significantly different from each other (Kruskal-Wallis one-way analysis of variance: P < 0.001). When the two types were considered separately sample sizes for the other taxa were too small for statistical analysis but means for P.m.gracilis (Type I x = 21.9 kHz; Type II x = 16.4 kHz and x = 33.7 kHz) appeared to be slightly different from all the others. P.m. nebracensis (x - 23.2 kHz and P.melanophrys (x - 23.0 kHz) were not significantly different from P.m.bairdi with their Type I squeaks, and P.difficilis and P.californicus were too few to determine differences.

Figure 3-8 Legend

Sound spectrograms of single squeaks compared with oscillograms of sets of squeaks. Time, as marked, on oscillograms. Spectrograms plot frequencies on the abcissa, db on the ordinate. (a), (b), and (c) 8-day old <u>P.californicus</u>, compare with Figure 3-7 a; (d) 4-day old <u>P.melanophrys</u>, the same individual as in Figure 3-7 b; (e) and (f) 8-day old <u>P.difficilis</u>, the same individual as in Figure 3-7 c; (g) and (h) 8-day old <u>P.m. nebracensis</u>, (h) is from the same individual as Figure 3-7 d.



FIGURE 3-8



Type II squeaks show much frequency and intensity modulation (Figure 3-9) although data were insufficient to identify particular modulation patterns with particular taxa or to determine the relationship between the modulations and the several strong frequencies. Figures 3-3, 4, 6, 7, 9 show the 2 strong frequency bands typical of Type II squeaks in comparison to the single bands of the Type I squeaks.

Sound spectra also clearly demonstrate the differences between the two types of squeak (Figures 3-5, 8). Sound pressure levels of more than a minimal 10 db above the baseline occur in discrete frequency bands. These freguencies were averaged within age periods for all the taxa and are presented in Table 3-2. Bands of similar frequencies resembling formants, are utilized by all the taxa, but different species show concentrations of sound pressure in different bands. Thus P.m.bairdi show a frequency band around 34 kHz with the strongest frequency typically 23 kHz, whereas P.polionotus sometimes shows a band at 25 kHz but emphasizes frequencies at 34 kHz. Mean frequencies for measurable bands are shown in Table 3-2, with the mean basic frequency (the strongest) underlined. Where no sound rose above the baseline a dash is used. The multiplicity of the frequency bands of P.m.bairdi and P.m.gracilis is clearly demonstrated as is the contrasting single strong frequencv of P.polionotus and P.leucopus.



Figure 3-9 Legend

Standard sonagrams of single distress squeaks compared with intensity contours of the same squeaks. (a) 5-day old P.m.bairdi; (b) contour of a; (c) 6-day old P.m. bairdi; (d) contour of c. The contours show a quavering quality in the intensity modulation and fluctuations of intensity greatest at the beginning and end of the squeak.



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FIGURE 3-9



Table 3-2

Distress squeak frequencies from 8 taxa of Peromyscus. Frequencies averaged for early (days 1 & 2), middle (days 3-10), and late (days 10 on) squeaks. Frequencies of greatest amplitude are shown; basic frequencies underlined. <u>n</u> equals numbers of squeaks analysed.

			В	and Numbe	r				
Day	n	1	2 -	3	4	5	6	7	8
P.polionotus									
Early	4	9.0		18.6(1)	25.7	36.8(1)		52.0	-
Middle	3	-	-	17.5	-	34.4	-	-	-
Late	2	-	-	-	-	33.8	-	-	66.0
P.leucopus Early	2	-	-	19.5	-	38.0	-	53.2	-
Middle	9	-	13.6	-	28.1	-	-	51.1	-
Late	2	-	-	-	-	30.0	-	-	60.0
<u>P.m.bairdi</u> Early	6	6.0	-	17.3	<u>21.3</u>	32.8	43.7	53.0	65.2
Middle	15	6.4	11.0	-	23.6	33.1	45.9	53.3	63.2
Late	4	-	11.0	16.8	23.7	34.2	45.1	52.0	66.6
P.m.gracilis	2	c 7	11 0	17 4	21 0	71 0		57 6	<u>۲0 с</u>
carly	<u>ت</u>	5.7	11.0	17.4	24.0	34.2	-	57.0	00.0
Middle	2 3	8.0 7.9	$12.0 \\ 12.8$	<u>19.1</u>	$\frac{22.8}{21.7}$	36.0 <u>38.8</u>	46.0 44.0	-	$68.0 \\ 61.0$
Late	1	-	10.0	-	20.0	-	40.0	-	60.0
P.m.nebracensis Middle	2	-	14.0	-	23.2	-	49.2	-	-
<u>P.melanophrys</u> Middle	2	_	11.0	-	23.0	-	40.8	-	66.8
<u>P.difficilis</u> Middle	1 2	- -	10.2 10.0	19.2	20.8	38.6	41.6	- 55.4	62.4
<u>P</u> . <u>californicus</u> Middle	1 2	$\frac{9.2}{9.4}$	-	$\frac{18.2}{18.5}$	28.0 26.8	34.0 36.0	48.0	56.0 56.0	-



The remaining 4 taxa provided too few data for strong comparisons but the measurements reinforced the observations made on Group A. <u>P.californicus</u> and <u>P.difficilis</u> show Type II squeaks, with frequency and intensity modulation (Figures 3-7,8). Squeaks of <u>P.californicus</u> are very long and often low in frequency (9.2 and 18.2 kHz; 18.5 and 36.0 kHz). <u>P. difficilis</u> shows frequencies similar to <u>P.californicus</u> but <u>P.m.nebracensis</u> and <u>P.melanophrys</u> are more similar to the <u>P.m.bairdi</u> and <u>P.m.gracilis</u>.

Species Differences - Temporal Parameters:

Numbers of squeaks per set, duration of individual squeaks, and duration of sets were called temporal parameters since they could be enumerated or measured in units of time. To answer the null hypothesis of no difference, in these parameters between taxa, two-way analyses of variance were done on daily means for each taxon in Group A, from day 1 until day 12.

Number of Squeaks per Set:

Daily means for Group A (<u>P.m.bairdi</u>, <u>P.m.gracilis</u>, <u>P.</u> <u>leucopus</u>, <u>P.polionotus</u>) are significantly different at P < 0.005 (parametric two-way analysis of variance). The difference between the means for <u>P.m.gracilis</u> and <u>P.leucopus</u> is significant at P < 0.01, and <u>P.m.bairdi</u> differ in mean number of squeaks from <u>P.polionotus</u> and <u>P.leucopus</u> at P < 0.05 (New Multiple Range test). <u>P.m.gracilis</u> do not differ significantly from <u>P.m.bairdi</u> or <u>P.polionotus</u> (Table 3-3).

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4 Temporal characteristics of the distress call of infant <u>Peromyscus</u>. Means for first taxa (Group A) are based on daily means for 12 days. These 4 taxa are significantly different from each other on all 3 parameters.

Species	ч	Squeaks per set x SE	Set_duration x SE	Squeak_duration x SE
Group A				
P.m.bairdi	78	3.70 ± 0.17	1.12 ± 0.11	0.18 ± 0.01
P.m. gracilis	73	4.05 ± 0.19	1.09 ± 0.12	0.15 ± 0.01
P. leucopus	75	3.15 ± 0.15	0.91 ± 0.05	0.17 ± 0.01
P. polionotus	72	3.70 ± 0.21	0.95 ± 0.09	0.13 ± 0.01
Group B				
P. californicus	12	4.48 ± 0.29	1.48 ± 0.17	0.18 ± 0.01
P.difficilis	9	3.79 ± 0.19	0.91 ± 0.05	0.15 ± 0.01
P.melanophrys	4	4.60 ± 0.51	1.57 ± 0.05	0.17 ± 0.01
P.m.nebracensis	N	4.28 ± 0.05	0.81 ± 0.21	0.14 ± 0*

*Analysis of variance not done on these data.

The mean number of squeaks in a set were not found to differ between the taxa in Group B (<u>P.californicus</u>, <u>P.dif-</u> <u>ficilis</u>, <u>P.melanophrys</u>, <u>P.m.nebracensis</u>) with a non-parametric analysis (Kruskal-Wallis), although larger sample sizes might have demonstrated a difference.

Set Duration:

The taxa in Group A differ in mean set duration at 0.05 > P > 0.025 (two-way analysis of variance). <u>P.m.bairdi</u> and <u>P.m.gracilis</u> differ significantly from <u>P.leucopus</u> and <u>P.polionotus</u>, but not from each other. <u>P.leucopus</u> and <u>P.</u> <u>poliontus</u> do not differ significantly. Group B taxa differ at 0.01 > P > 0.011 (Kruskal-Wallis). <u>P.californicus</u>, and <u>P.m.nebracensis</u> have longer sets of squeaks than <u>P.diffi-</u> <u>cilis</u> and <u>P.melanophrys</u>.

For the taxa in Group A the numbers of squeaks in a set is significantly correlated with the duration of the set (Spearman-Rank Correlation Coefficient; P.m.bairdi, P.m. gracilis, P.polionotus, P < 0.01; P.leucopus, P < 0.05). Although the inter-squeak intervals were not measured, systematic sample measurements indicated that they are fairly invariable and average about 0.1 sec. for all taxa. When species means were considered over all 8 taxa a significant correlation was found between the number per set and the duration of the set, also (Spearman-Rank Correlation Coefficient; 0.05 > P > 0.01) but there is no correlation between the number of squeaks per set and the duration of the squeaks.



Squeak Duration:

The 4 taxa in Group A differ significantly in mean squeak duration at P < 0.005 (two-way analysis of variance). By the New Multiple Range test <u>P.m.bairdi</u> and <u>P.polionotus</u> were shown to differ at P < 0.01; <u>P.leucopus</u> and <u>P.polio-</u> <u>notus</u> differ at P < 0.05. <u>P.m.bairdi</u> and <u>P.m.gracilis</u> do not differ from each other nor from <u>P.leucopus</u>, and <u>P.m.</u> gracilis does not differ from P.polionotus.

The species in Group B do not differ significantly in terms of mean squeak duration ($\underline{P}.\underline{m}.\underline{nebracensis}$ had too few means to include in the analysis).

Although these 8 taxa do not differ significantly on every parameter they all differ from the others on at least one measure. For example, in Group A, P.m.bairdi and P.polionotus have similar numbers of squeaks per set but significantly different squeak durations and frequencies. The squeaks of P.leucopus are of about the same duration as those of P.m.bairdi but there are fewer P. leucopus squeaks in a set and P.leucopus sets are shorter in duration than those of P.m.bairdi. P.m.gracilis has the greatest mean number of squeaks per set of the taxa in Group A, but they are of short duration. Comparing these with the 4 taxa in Group B, P.melanophrys has the largest mean number of squeaks per set and the longest set duration but P.californicus and P.m.bairdi have the longest squeaks (Table 3-3). P.polionotus has the shortest squeaks, P. leucopus has the fewest squeaks per set, and P.leucopus and



<u>P.difficilis</u> have the shortest sets, while <u>P.polionotus</u> has the highest mean basic frequency Tables 3-2, 3).

Age Differences - General Observations:

It was hypothesized that as a mouse pup develops it gains increased control over its vocal apparatus. As it begins to receive some feedback from the sounds it produces it becomes better able to modify the sound. Ontogenetical changes were thus expected to be apparent in the squeaks. Observations and statistical analyses of the data confirm this hypothesis. General patterns of change were found to be similar across all the taxa analyses, with major changes in frequency and temporal parameters between day 2 and days 3 or 4 and again at about day 10 (Table 3-2, 4; Figures 3-10 to 3-17). The most stable period for all parameters, where they change most slowly and show least variation was found to be the middle period between 3-4 and 10 days of age.

Age Differences - Frequencies:

Squeaks recorded on day 1 and most of those from day 2 showed wide variation in frequency; all of these frequencies were usually lower than the mean frequencies characteristic of the stable middle period (Table 3-4; Figures 3-12, 13, 14). Some squeaks recorded on day 2 and all the squeaks recorded on day 3 or later show little variation around this "species-characteristic" frequency. Records were too few in number and too far apart (24 hours) to determine



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Figure 3-10 Legend

Sonagrams of single squeaks. (a) 1-day old <u>P.m.</u> <u>bairdi</u>, time mark = 0.05 seconds; (b) to (e) time marks = 0.1 second, (b) 13-day old <u>P.m.bairdi</u>, distress cry; (c) 13-day old <u>P.m.bairdi</u>, pain squeak displaying multiple harmonics and great frequency modulation in a much shorter squeak than the distress cry; (d) 8-day old <u>P.m.</u> <u>bairdi</u>, distress cry; (e) 13-day old <u>P.m.gracilis</u>, distress cry.







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Figure 3-11 Legend

Oscillograms and sound spectrograms for P.maniculatus bairdi pups of different ages. Compare with sonagrams of same species in Figure 3-10: (a), (b), and (c) 1-day old P.m.bairdi, the same individual as in Figure 3-10 a. The squeak (b) is the first of the set shown in (a), while (c) is the second squeak; (d) and (e) squeaks from a 13-day old pup, compare with sonagrams from the same individual in Figure 3-10 b; (f), (g), and (h) pain squeaks from a 13-day old pup, the same individual as in Figure 3-10 c. Although the bursts in (f) appear to be of almost normal length they are actually 2 or 3 squeaks run together, as shown in Figure 3-10 c. Squeaks displayed in (g), (h) show energy present at a wide band of frequencies in these squeaks. (i) and (j) squeaks from an 8-day old pup, (i) is from the same individual as Figure 3-10 d.


FIGURE 3-11

Table 3-4

Distress squeak frequencies (daily means in kHz) from Peromyscus maniculatus bairdi pups of selected ages. The frequencies of the greatest amplitudes, equivalent to possible formants, are shown; basic frequencies underlined. \underline{n} equals numbers of squeaks.

Band Numbers									
Day	n	1	2	3	4	5	6	7	8
Early									
1	2	6.0	-	-	20.8	-	40.8	-	-
2	4	-	-	18.2	-	35.8	48.0	53.0	65.2
Middle	3								
3	3	-	-	-	26.7	-	-	54.2	-
6	3	-	11.2	16.8	26.0	-	46.0	50.4	62.8
7	4	7.4	11.3	-	23.1	-	40.0	5044	63.0
8	2	6.2	10.4	-	22.0	31.2	47.2	52.0	63.2
10	3	6.6	11.1	-	22.2	34.0	44.6	56.0	64.0
Late									
12	2		10.7	16.8	24.0	34.4	45.2	52.8	-
13	1	-	11.2	-	22.0	-	44.0	-	66.6
14	1	-	11.2	-	24.8	36.0	46.0	50.4	-

whether the change in frequency is gradual or as sudden as it appears to be. Typically the shift in frequency was recorded on the day corresponding to the mean date of pinnae erection (Layne, 1968). One record of an unusual high frequency for a 2-day <u>P.polionotus</u> pup, came from an animal not observed to have its pinnae up, although the ears might have been unsealed from the head or the nerves newly capable of receiving sound through the skull.

From day 3 on variations observed were slight (Tables 3-2, 4). Frequencies appeared to remain at a level characteristic of each species (Table 3-4, Figures 3-5, 7, 8). Towards day 10 most squeaks lack visible fundamentals and become increasingly pure tones (Tables 3-2, 4; Figures 3-10 to 3-14).

In order to compare data from <u>P.m.bairdi</u> and the other taxa, daily means for major frequencies were grouped into <u>early</u> - days 1 and 2, before the average date of pinnae erection; <u>middle</u> - days 3-10, after pinnae were up but before eyes began to open; and <u>late</u> - after day 10, when eyes and external ears were increasingly ready to open, homothermy developing, and squeaks less frequent (Table 3-2, 4). Such groupings made frequency comparisons much more realistic than single average frequencies for each taxon, although total sample sizes were reduced, often below the levels required for accurate statistical comparisons.

Daily means for frequencies for <u>P.m.bairdi</u> are shown in Table 3-4. The mean basic frequency is lowest for day 2,



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. . three of the four squeaks sampled on that day having low frequencies not seen on other days. The mean basic frequency is highest on days 3 and 4 (Figures 3-11, 12). Only on days 1, 7, 8, and 10 are possible fundamental frequencies present. Squeaks recorded on days 2, 6, and 12 show an intermediate frequency, similar to those visible in many records in <u>P.m.gracilis</u>, <u>P.difficilis</u> and <u>P.cali-</u> fornicus (Figures 3-5, 8, 10, 12).

Results of the multiple sonagrams and spectrograms made of P.m.bairdi squeaks for different ages demonstrate changes in temporal parameters and frequencies and provide a basis for comparison with the data from the other taxa, particularly those in group A. Visible frequencies of a 1-day old <u>P.m.bairdi</u> show high, pure tones (about 20 kHz) with sharp onsets and offsets (Figure 3-10a). By the middle period, squeaks of an 8-day old pup display more energy at a second harmonic (Figure 3-10b). Spectrograms of more squeaks from the same individuals emphasize the trend to increasing complexity and increasingly invariant patterns with less frequency modulation (3-12a,b,i-n). Maximum complexity is seen in the "pain" squeak with its many strong frequencies (Figures 3-10c, 11f, g,h).

<u>P.m.bairdi</u> Type II squeaks do not show as much energy in the second frequency as those of <u>P.m.gracilis</u> or <u>P.</u> <u>californicus</u> but rather a broader band and greater frequency modulation for both strong frequencies (Figure 12f, h), clearly different from Type I squeaks (Figure 3-12e, g, i-1).

Figure 3-12 Legend

Oscillograms and sound spectrograms for additional distress squeaks from P.m.bairdi pups, showing ontogenetical changes and differences between Type I and Type II squeaks. (a) and (b) 2-day old pup, the first frequency peak in the spectrogram at (a) actually corresponds to the lower frequency at the end of the squeak. (c) and (d) 6day old pup; (c) shows a Type I squeak with a higher basic frequency than average but a purer tone than in (a). (e), (f), (g), and (h) 4 squeaks, in order, from one set emitted by a 7-day old pup. (e) and (g) are examples of Type I squeaks, while (f) and (h) are examples of Type II squeaks with more overtones present and lower basic frequencies. Notice that all four have a virtually identical overtone present at 43.2-44 kHz. (i) and (j), 2 squeaks from different 10-day old pups, aligned to show similarities. (k) and (1) 2 squeaks from different 12-day old pups, also aligned to show similarities. A strong increase in basic frequency is apparent. (m) squeaks from 13-day old pup showing visible complexities of intensity and frequency modulation. (n) a very clear tone from a 14-day old pup, here a fundamental is present for part of the squeak.



FIGURE 3-12

After day 10 only a few major frequencies are emphasized and the squeaks give the appearance of little frequency modulation.

<u>P.m.gracilis</u> shows very similar patterns to <u>P.m.</u> <u>bairdi</u> with major differences in the greater prevalence of Type II squeaks. Frequency modulation is much stronger in the <u>P.m.gracilis</u> squeaks than in the squeaks of the other taxa recorded. Even squeaks from the 1-day old pup show strong modulation (Figure 3-13a). A few recordings show pure tones of Type I squeaks (Figure 3-13,c) but the majority of the samples showed the multiple harmonics and the frequency modulations of the Type II squeaks (Figures 3-3a, b; 3-4d; 5g,h; 10e). Mean basic frequencies for <u>P.m.gracilis</u> Type II squeaks increase with age from 11.8 kHz and 24.8 kHz in the early period to 19.8 and 38.8 kHz and decrease for Type I squeaks, from the middle to late periods; 22.8 kHz - middle to 20.0 kHz - late.

Pain squeaks of <u>P.m.gracilis</u> are virtually indistinguishable from those of <u>P.m.bairdi</u>, being short squeaks, in continuous groups with multiple strong frequencies (Figures 3-10c, 11f, g, h). Often very short squeaks are fused into a short chirp ($\bar{x} - 0.1$ sec.).

Data for <u>P.polionotus</u> and <u>P.leucopus</u> demonstrate similar ontogenetical trends. These two species differ from the <u>P.maniculatus</u> mice in showing no Type II squeaks and little frequency modulation. In both, frequencies rise and become more pure with age (P.leucopus Figures 3-14a, e, f,



Figure 3-13 Legend

Oscillograms and sound spectrograms from <u>P.manicu-</u> <u>latus gracilis</u> pups; distress squeaks for several ages and pain squeaks. (a) 1-day old pup; (b) 8-day old pup, showing a clearer frequency than (a); (c) and (d) 10-day old pup, 2 types of squeaks. (c) is a Type I squeak with a single high frequency and one harmonic and (d) is a Type II with almost equal sound energy at two frequencies; (e) and (f) normal distress squeaks from 13-day old pup, the same individual as in Figure 3-11d, the squeak in (f) is a Type I squeak; (g) oscilloscope trace of pain squeaks from a 14-day old pup; (h) and (i) spectrograms of single pain squeaks from the same individual. These squeaks show broad frequency bands similar to the <u>P.m.bairdi</u> pain squeaks in Figure 3-11g and h.





Figure 3-14

Legend

Sound spectrograms and oscillograms comparing <u>P</u>. <u>leucopus</u> with <u>P.m.bairdi</u> and <u>P.polionotus</u> across ages. Time as marked. (a) <u>P.leucopus</u>, l-day old; (b) <u>P.polio-</u> <u>notus</u>, l-day old; (c) <u>P.m.bairdi</u>, 3-day old; (d) <u>P.polio-</u> <u>notus</u>, 3-day old; (e) and (f) <u>P.leucopus</u>, 3-day old, note basic frequency identical to <u>P.m.bairdi</u> in (c); (g) and (h) <u>P.leucopus</u>, 7-day old, same individual but time base 1/2 that in (f); (i) 9-day old <u>P.leucopus</u>; (j) 12-day old <u>P.leucopus</u>. As the pup ages there is a general tendency towards sharper frequencies with fewer visible harmonics.



FIGURE 3-14



6c, 3b, 4a; P.polionotus Figures 3-6b, 4b, 5d, 14b, d).
P.polionotus squeaks are typically sharper, shorter and
higher than those of P.leucopus.

Age Differences - Temporal Parameters:

Numbers of Squeaks per set:

Significant differences were found over age among Group A taxa (0.01 > P > 0.005; two -way analysis of variance). Numbers of squeaks per set rise to a maximum for <u>P.m.bairdi, P.polionotus and P.leucopus</u> on day 2, and maintain a level until day 10. At this point <u>P.leucopus</u> and <u>P.m.gracilis</u> increase the number while <u>P.m.bairdi</u> and <u>P. polionotus</u> precipitously decrease. <u>P.m.gracilis</u> starts on day 1 with a high number of squeaks, increases to day 2, slowly decreases to day 14 and then increases again. (Figure 3-15).

Set Duration:

Mean set duration differed significantly over age for the 4 taxa in Group A (P < 0.005; two-way analysis of variance). Patterns of change resembled those of numbers of squeaks per set, except that <u>P.m.gracilis</u> does not show a rise in duration after day 14 (Figure 3-16).

Squeak Duration:

The taxa in Group A differed significantly over age for squeak duration (P < 0.001; two-way analysis of variance). Squeaks tended to start short, lengthen to a plateau on day 3 or 4 and to remain fairly constant until day 10, at which



Figure 3-15 Legend

Number of squeaks per set: Daily mean numbers of squeaks in a group plotted against age of pups. Solid line = $\underline{P}.\underline{m}.\underline{gracilis}$; dashed line = $\underline{P}.\underline{m}.\underline{bairdi}$; diamonds = $\underline{P}.\underline{polionotus}$; elipses = $\underline{P}.\underline{leucopus}$. Solid arrows indicate predicted age of ear opening, as demonstrated by a startle response. Open arrows indicate predicted age of eye opening. Circles on lines indicate estimated age at erection of pinnae.







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Figure 3-16 Legend

Set duration: Mean duration, in seconds, of sets of squeaks, plotted against age of pups. Solid line = <u>P.m.gracilis</u>; chain = <u>P.m.bairdi</u>; diamonds = <u>P.polionotus</u>; elipses = <u>P.leucopus</u>. Arrows and circles, as in Figure 3-15 indicate the estimated ages at eye and ear opening and pinnae erection.





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point they decrease (Figure 3-17). <u>P.m.gracilis</u> shows an erratic lengthening and shortening of squeaks after day 12. All the parameters show the abrupt changes about day 3 and after day 10, approximately the days when the pinnae become unsealed and the eyes and external ears open.

Discussion:

The observations and analyses reported in this chapter demonstrate the physical differences which exist between the distress squeaks of pups of the various taxa examined. Typically <u>P.maniculatus</u> pups show major energy peaks between 20 and 24 kHz. Some of the subspecies, particularly <u>P.m.gracilis</u>, produce Type II squeaks with several strong peaks of energy while other subspecies, <u>P.m.</u> <u>bairdi</u>, more usually produce Type I squeaks. Fundamentals are often visible, particularly with the youngest pups and at all times the frequency should make the pitches of squeaks of the other species perceptibly different to these mice.

On temporal parameters the three subspecies of \underline{P} . <u>maniculatus</u> do not show clear relationships; <u>P.m.nebracensis</u> being different on all three parameters (set duration, number of squeaks per set, squeak duration) from the other two and <u>P.m.gracilis</u> and <u>P.m.bairdi</u> similar only in the duration of sets. <u>P.m.bairdi</u> has longer squeaks than <u>P.m.gracilis</u> but fewer of them, while <u>P.m.gracilis</u> has many shorter squeaks. Related vocal musculature may explain similar



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Figure 3-17

Legend

Squeak duration: Mean duration of distress squeaks, in seconds, plotted against age of pups. Solid line = $\underline{P} \cdot \underline{m}$. gracilis; chain = $\underline{P} \cdot \underline{m} \cdot \underline{bairdi}$; diamonds = $\underline{P} \cdot \underline{polionotus}$; elipses = $\underline{P} \cdot \underline{leucopus}$. As in preceding figures (3-15, 3-16) arrows indicate estimated ages of ear and eye opening and circles indicate age at pinnae erection.



Squeak Duration



frequencies while differential emphasis on frequency bands (formants) components may provide sufficiently divergent pitches for specific identification by the mice. Temporal components may not be as important as pitch in recognition by the mice.

P.polionotus is a member of the P.maniculatus species group, P.leucopus is grouped in the same subgenus (Hooper, 1958) and both may be sympatric with P.maniculatus in areas of the Eastern United States. The P.leucopus and P.maniculatus mice used in this study were from populations which were sympatric in some areas. At the same time the mean basic frequencies of P.leucopus and P.polionotus differ the most from frequencies of the other species. Both P. leucopus and P.polionotus have high squeaks (overall mean basic frequency 33.1 kHz, for P.polionotus; 32.1 kHz, for P.leucopus) and the two species have similar numbers of squeaks per set and set durations. P.leucopus has longer squeaks than P.polionotus. On 3 of the 4 major squeak characteristics measured, P.leucopus diverges from P.m. bairdi and on 4 of 4 diverges from P.m.gracilis. P.polionotus is similarly different from P.maniculatus mice.

The other 3 species of mice tested are completely allopatric and more distantly related. No particular patterns of characteristics emerge except that all show frequency patterns similar to the <u>P.maniculatus</u> mice. On temporal characteristics <u>P.difficilis</u> most resembles <u>P</u>. leucopus. P.californicus and P.melanophrys are alike on



all measures except frequency, where they differ slightly.

The original supposition that frequency patterns would be more conservative to change than the temporal patterns appears to be supported by the similarities in frequency found in the <u>P.maniculatus</u> mice. Phylogenies might be based on sounds with more data on additional species and local populations.

A reduction in variation and a general refinement of a typical pattern show up in the comparisons of the squeaks from pups of increasing age. As expected, squeaks do become shorter and less frequent as the pups grow older. In addition, frequencies tend towards purer tones, a change which must carry with it some change in pitch.

Temporal and frequency characteristics do not change at a constant rate but show the greatest variation in the several days before the external pinnae are fully extended and around the time of ear and eye opening. Although pups do not respond to loud sounds before the 12th to 13th day when their ears open (Huff pers. obs., 1968; Layne, 1968), major changes in temporal characteristics and frequency occur on about the third day after birth. It may be that the ear starts to function at this point and the pup begins to get some auditory feedback through bone conduction at least.

More gradual changes in set duration and the number of squeaks in a set do occur and may be related to decreasing motivation to squeak, culminating in reduced or absent

squeaks at the time of eye opening. The auditory cues to the mother thus become weaker stimuli correlating with her changing hormones and resulting in diminished motivation for search and retrieval.

Although each of the 8 taxa recorded differed significantly from each of the others on at least one of the 4 major parameters no correlation was found to exist between rankings on means of any of the parameters and overall body size. P.polionotus, the smallest mouse recorded, has the highest mean basic frequency but P.leucopus, a middle-sized mouse, can produce even higher frequencies at the early stages. P.californicus, P.difficilis and P.m. gracilis, all large mice, have equally high frequencies in their Type II squeaks. P.polionotus has the shortest squeaks but P.m.bairdi, P.difficilis, and P.leucopus have the shortest sets, and P.leucopus has the fewest squeaks per set. Frequency is the parameter most likely to vary with size but the mice recorded may not be divergent enough in size to have functionally different vocal cords. Size of pups or of actual larynxes may correlate better with mean basic frequency.

Changing frequencies and multiple strong frequency bands probably make the pitch of squeaks of pups of various ages and species perceptibly different to the mice. When recording tapes were slowed by a standard amount (15 ips to 1-7/8 ips) it was possible for human ears to hear pitch and pattern differences. The relative importance of pitch



and the temporal parameters in information transfer for the mice cannot be precisely assessed from the data presented here. It might be suggested that both would be important as elements of redundancy in the information transfer, with frequency carrying more subtle cues on age, species, and perhaps individual identity.

Efficient localization of the source of a distress squeak by a mother mouse depends on her detection of differences of intensity, phase and time of arrival of the sound at her two ears. Localization by means of intensity is most effective when the head is wider than the wavelength of the sound and can block the sound from directly reaching one ear or the other (Marler, 1961, 1967). Localization by means of phase differentials is best when the sound reaches both ears directly but out of phase, when the wavelength is longer than the width of the head between the ears. Abrupt discontinuities and transient frequencies permit localization by comparison of the arrival times at the two ears.

This last characteristic is apparent in all the distress squeaks recorded, although lacking in the pain squeaks. Sharp onsets and offsets and grouped short squeaks provide ample discontinuities for time comparisons and thus localizations of distress squeaks by means of time differences.

Measurements of the widths of the skulls of the mothers of the pups in this study were not made, but rough estimations were made on the basis of skull measurements


given for <u>P.m. gracilis</u>, <u>P.m. bairdi</u> and <u>P. leucopus</u> in Burt (1957). Width measurements for skulls used in that source were made on distance between zygomatic arches. Ear to ear measurements made on living mice with calipers agreed, in general, with these data. The following table collects frequency data and estimates head width for the 3 taxa.

Table 3-5

Comparison of estimated ear-ear head width measurements of adult mice of 3 taxa of <u>Peromyscus</u> with mean basic frequencies of distress squeaks of pups of the same taxa.

Species	Mean width zygomatic arch(mm)	Mean width ear-ear(mm)	Wavelength of mean basic frequency (mm)
<u>P.m. bairdi</u>	12.0	11.7	early 15.9 middle 13.8 late 13.8
P.m. gracilis	13.1	-	early 27.6 & 13.3 middle 17.4 & 8.5 late 16.6
P. <u>leucopus</u>	12.6	12.7	early 8.7 late 11.8 late 11.1

As the measurements of head width on the living mice might differ slightly from skull measurements due to the presence of fur, skin, and pinnae, the effective width would be expected to be somewhere lower than the estimated ear-ear measurement and the zygomatic area measurements.



Since the wavelengths of the mean basic frequencies are longer than the estimated head widths of the relevant hearers for P.m. bairdi these mice would be able to localize the sources of the sounds by means of phase differentials. Wavelengths for frequencies for P.m. gracilis are also longer than the estimated head width with the exception of the short mean for the Type II squeaks for the early and middle age groups. If these high frequencies are heard as separate waves P.m. gracilis might be able to utilize both localization methods, phase differences and differences in intensity. All of the wavelengths calculated for mean basic frequencies for P. leucopus are shorter than the maximum head width. It is thus possible that these mice could also use both phase and intensity differences but more precise measurements of living mice are needed for a definitive answer. All of the species considered have at least two methods of accurately locating the sounds produced by their pups. Redundancy provided by the repetition of the squeaks would improve efficiency.

Pain squeaks do not provide easily localizable characteristics and are analagous to the warning calls of birds which alert others to danger but are difficult for a predator to locate. The pain squeaks recorded for the <u>P</u>. <u>maniculatus</u> mice were very similar between the two sub. species, with gradual onsets and offsets, vague intensity modulation and multiple frequencies. Intensity differences, phases and times of arrival would be almost impossible to



distinguish. In addition multiple repetitions of these very short squeaks would insure that the information was transmitted.

The observations based on the analyses of these squeaks suggest that the obvious characteristics of the sounds have important functions in the communication systems of these animals. Even greater subtleties of information transfer probably remain to be explicated by further analyses and experimentation.

CHAPTER 4

Effectiveness of Auditory Stimuli in Eliciting Maternal Searching: Activation Test.

Schleidt (1956), Noirot (1960; 1964c), Sewell (1970) and others have mentioned that mouse pups which do not squeak are not retrieved. In pilot studies, lactating <u>Peromyscus</u> <u>maniculatus</u> rarely left their pups and nests to explore the experimental chamber unless they heard a squeaking pup or a recording of one. The following experiment was designed to test the hypothesis that the squeaks of mouse pups out of their nests stimulate a mother mouse to search for the sound source. The pilot studies suggested that this is a sufficiently strong stimulus to cause a lactating female to leave her nest and nursing pups and enter a relatively strange, open arena. Without this stimulus a mouse might spend most or all of the daylight hours in her nest with her young (King, 1963).

Subjects:

Forty-two lactating <u>Peromyscus</u> <u>maniculatus</u> <u>bairdi</u> first generation daughters of wild-caught parents were used as the experimental animals in 3 groups of 14 mice each. All the females were between 6 and 18 months of



age. Each mother mouse was assigned to one of the three experimental groups, in sequential order, as her litter was born. Those females which cannibalized their young before the test were not used in the experiment. All the litters were randomly culled to four young one day after birth. The females were maintained with their mates and litters in standard colony cages in the mouse colony room until 1-3 days prior to testing. At this time the females and their litters were moved into a room adjacent to the test chamber.

Experimental Apparatus:

During the pretest and test days each mother and her litter was housed in a modified colony cage which had a 1-1/2 in opening in the lower center of each end. These openings were glued to plexiglas tunnels (2" x 2") and until test time they were both blocked with guillotine doors. The plexiglas tunnels connected the cage to two square (2' x 2' x 18" high) plywood arenas (Figure 4-1). These bottomless arenas were placed on the vinyl-tile floor and were covered with 1/4 in screen. The entire Sound Choice Box (SCB), with the exception of the mouse cage and tunnels, was painted flat black. At each end of the SCB, opposite the tunnel entrance, a screened opening (8" x 6" high), covered with black cloth, concealed a high-frequency speaker (University Sphericon Tweeter). As diagramed in Figure 4-1, photocells placed beside the





Figure 4-1 Legend

Sound Choice Box. A mouse placed in the central cage could be exposed to sounds presented from either speaker (S) or both simultaneously. Subsequent activity could be monitored automatically by means of photocells (P) triggered by lights (L) placed on the opposite side of the apparatus. Each arena is an open field 24 inches square. Photocells are placed at critical position assessment points rather than equidistantly.





SCB arenas and the tunnels and connected to an Esterline-Angus Event Recorder monitored the movements of the mouse in the apparatus.

The Sound Choice Box and speakers were placed in a sound-damped room and connected by cables to the tape recorder, amplifiers, and event recorder in an adjacent room. From 0600 to 1800 hours the test room was illuminated by a 60-watt light bulb suspended from the ceiling and the rest of the time only by the six 10-watt red bulbs used with the photocells.

Test Sounds:

The three sounds used in this test were recorded with the same equipment and procedures as the squeaks for analysis (Chapter 3). Sounds were recorded for five minutes on one tape segment, each on a separate channel. The squeaks of a five-day-old <u>P.maniculatus bairdi</u> were used as a test sound and compared to "white" noise from radio static and mechanically generated clicks. The "white" noise was presented as a control for reactions to possibly intermittent speaker hiss and the mechanical clicks were presented as a control for responses to sounds having some similar characteristics to the squeaks. Since all of the sounds were on one segment of tape it was possible to present them simultaneously, in any pairing desired, by plugging the appropriate channel into the speaker.

Each of the three experimental groups was presented



with a different combination of the sounds. The "squeakcontrol" group heard the recorded mouse squeaks and the control "white" noise, the "click-control" group heard the clicks and the "white" noise, and the "choice" group heard the squeaks versus the clicks. In this way the mice were tested for their responses to the mouse squeaks alone, for responses to a temporally similar sound alone, and for their choices between the mouse squeak and the partial copy, differing primarily in frequency. That sound to which each group was expected to respond was defined as the "correct" stimulus. Thus for the first group, squeak-control, the "correct" stimulus was the mouse squeaks, for click-control, it was the clicks, and for the choice group, the mouse squeaks were defined as "correct" and the clicks as "incorrect."

Procedure:

Each mouse was placed with her 4-6 day old litter in the central cage in the Sound Choice Box on the afternoon of the day preceding the test day. She was permitted free access to both arenas for the 22-24 hours prior to the test but her activity was monitored by means of the photocells and event recorder. The activity records were examined before testing in order to discover preferences for one arena over the other. Where preferences appeared, the initial test sound was presented from the non-preferred side, otherwise initial presentations were random.

Immediately before testing, the tunnel doors in the



central cage were closed and the mouse pups were removed to another room. Then the doors were raised and the mother was permitted to search for them. The length of time until she returned to the nest and remained there for one minute (as indicated by the activity record) was scored as the "latency-to-calm."

Once the mother mouse had remained in the nest for one minute, the test sound was presented at one end of the SCB. The taped sound was played continuously until the mouse entered one of the tunnels or until five minutes had passed. If the mouse responded the sound was continued for only one more minute.

Most females returned to their nests within one minute after the sound had been turned off. All were allowed a one minute intermission between the time they returned to their nests and the next presentation of sound. In the second trial the test sound was presented from the opposite speaker. These reversals could be accomplished without entering the experiment room by alternating the speaker connections to the recorder. The protocol for the second, third and fourth trials was identical and each time the test sound emanated from the alternate speaker. The testing protocol can be outlined as follows:



	cage clos	sed \rightarrow litter out \rightarrow cage opened	
	"t:	ime-to-calm" > 3 minutes	
Arena A		t	Arena B
		l minute intermission \downarrow	
sound	<u> </u>	5 minutes test ↓	control
		l minute intermission \downarrow	
control	<	5 minutes test \downarrow	sound
		l minute intermission \downarrow	
sound	<	5 minutes test \downarrow	control
		l minute intermission \downarrow	
control	<	5 minutes test	sound
		litter returned	

Variables:

The independent variable was the sound presented. All other factors which might have influenced the response of the mouse, such as age, sex, experience, reproductive state, age of own litter, and some attributes of the stimulus were held as constant as possible.

The Sound Choice Box was designed to permit measurement of the dependent variables of response latency, direction of choice, number of choice changes, and the distance traveled to or from the stimulus. Latencies were measured in seconds with a stopwatch, from the time the sound was turned on until a recording pen clicked as the mouse moved into the beam of a photocell. These measurements were



compared for accuracy against the same time measured by the event recorder. Choices of direction were indicated on the chart of the event recorder. Distance traveled by the mouse was recorded as the farthest photocell triggered in any excursion from the nest.

The experiment was planned so that parametric or nonparametric analyses of variance could be done on the test averages for the subjects in each group. Comparisons could be made between groups for all variables and within groups between numbers of "positive" and "negative" responses, and distances to the different sounds.

Results:

The dependent variables in this test can be considered to fall into two categories: variables related to general activation and those related to preference for one sound over another. Response latencies and total number of responses were measures of activation, while numbers of "positive" responses, reversals, and distances traveled in particular directions were measures of preference. Two variables related to the homogeneity of the experimental groups will be reported first as they support the contention that the experimental groups were similar in composition. Litter Age

In order to ensure a relatively homogeneous reproductive state among the experimental animals and a similar level of responsiveness to pups the mice were tested when



their litters were about six days old. Since any one mouse occupied the test apparatus for 24 hours prior to the actual test, logistics occasionally demanded testing a mouse with a litter that was one or two days older or younger than six days. Nevertheless the mean litter age did not differ significantly across groups (one-way parametric analysis of variance) and the variances were homogeneous (Table 4-1). Latency-to-Calm

Once the mice had been established in the SCB and their litters removed, the length of time required for them to cease searching, to return to their nests, and remain there for at least one minute was measured. It was expected that this latency indicated the level of arousal of the mouse after the disturbance of the removal of the pups. Although some mice might be more excited and more active following this treatment than others, the experimental groups should not differ significantly in latency since this procedure occurred prior to the experimental treatment. All of the mice took at least three minutes (counting the one minute in the nest) to reach the desired level of calm and there were no significant differences in mean latencies between the groups (Table 4-1).

Total Number of Responses

The total number of responses were defined as the numbers of trials in which the mouse responded at least once; the highest total a mouse could have was four. If the mouse changed its direction and went to the other side



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Measures of homogeneity of reproductive state and responsiveness of <u>Peromyscus</u> <u>maniculatus</u> mothers in three experimental groups: Litter age (days) and <u>maniculatus</u> methenoy-coalm (seconds after 3 minutes).

		Litter Age	Latency-to-calm
Groups	ц	x S.E.	x S.E.
Squeak-control	14	6.5 ± 0.31	240.79 ± 55.42
Click-control	14	6.4 ± 0.31	284.71 ± 83.61
Choice	14	6.6 ± 0.29	267.32 ± 84.94

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of the box, the switch counted as a reversal. A complete response was scored if the mouse only triggered the photocell in the tunnel between the cage and an arena.

Heterogeneity of the variances required a nonparametric statistical test (the STP) to evaluate the differences between the mean numbers of responses. The groups did not vary significantly in the mean number of trials in which there was a response (Table 4-2). However, the variation in the means was much lower in the choice group, indicating that more mice in that group responded in at least three trials than in the other groups.

Number of First Responses Correct

The mean numbers of first responses which were to the correct side, i.e., to the squeaks for the squeakcontrol and choice groups and to the clicks for the clickcontrol group, differed significantly among the groups (parametric one-way analysis of variance 0.05 > P > 0.01). By the New Multiple Range test the greatest part of the difference was found to be between the click-control and the choice groups (Table 4-2), at the 1% level.

More mice in the squeak-control and choice groups directed greater than 50% of their first responses to the mouse squeaks than would have been expected by a hypothesis of random response. The preferences in these two groups were significant by the chi-square test (P < 0.01 and P < 0.01 respectively).



Table 4-2

Mean responses and mean response latencies of <u>Peromyscus maniculatus</u> mothers to <u>P.maniculatus</u> distress gueaks and control sounds in mothers to <u>P.maniculatus</u> distribution test

	Squeak - control	Click - control	Choice	д
ч	14	14	14	
Trials with a response	3.29	3.64	3.93	n.s.
Number first responses correct	2.43	2.07	3.14	0.05
Latency to first response (seconds)	54.34	113.13	77.09	10.0
Latency to correct response (seconds)	100.13	117.32	113.30	0.05

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Latency to First Response

The time from onset of the test sound until the mouse triggered either of the tunnel photocells was defined as the latency to first response. Variances in these latencies were quite large and the data skewed to the left, so a non-parametric Kruskal-Wallis one-way analysis of variance was used. This test showed that the groups did differ in latency (0.01 > P > 0.001). The squeak-control group had the shortest mean latency to respond and the click-control group took the longest time to make any responses (Table 4-2).

Latency to Correct Response

This was the measure of the time from the onset of the test sound until the mouse triggered the tunnel photocell on the side of the SCB where the "correct" sound was being played (defined as the mouse squeaks for the squeakcontrol group and the choice group, and the clicks for the click-control group). The variances in latency were again quite large and the data negatively skewed so the Kruskal-Wallis test was used. Although the means of these three groups were closer when correct responses were considered than when any responses were considered they were nevertheless significantly different at the 5% level (0.05 > P > 0.02). Again the squeak-control group had the shortest latency and the click-control the longest.

Distance

The distance a mouse moved in either arena was



measured by the photocells triggered as the mouse passed. It was impossible to tell whether the path of the mouse was straight from one photocell to the next or composed of numerous advances, retreats, and excursions from one side of the box to the other. Observation showed that both types and all sorts of intermediate variations occurred. In order to avoid "losing" the mouse, the photocells were positioned at critical points in the SCB; near the speakers, across the entrance to the arenas, and across the tunnels between the arenas and the cage, rather than equidistantly. Since they thus measured not absolute distance, but just the number of times the mouse passed by, the results are tabulated in the form of scores. Each photocell was numbered by its position relative to the central cage; the number ones crossed the tunnels, number twos caught the mouse just entering the arenas, and the number threes were nearest the speakers. A mouse that ran all the way to the speaker in each trial would accumulate a mean score of 3 while a mouse which never did more than stick its head into the tunnel would have a mean score of 1 (Table 4-3).

Distance Score to Correct Response

These data showed homogeneity of variance so the null hypothesis that the groups did not differ was tested by a parametric one-way analysis of variance. The groups were found to differ significantly overall (P < 0.05). By the New Multiple Range test the click-control group



Table 4-3

Mean distance scores and mean choice reversals of <u>Peromyscus maniculatus</u> mothers responding to distress squeaks of <u>P.maniculatus</u> pups and control sounds

	Squeak-control	Click-control	Choice	д
ц	14	14	14	
Distance correct response	2.33	1.85	2.55	0.05
Distance incorrect response	1.73	2.14	0.73	0.01
Choice reversals	2.43	. 3.29	1.93	0.05

differed from the squeak-control group at P < 0.05 and from the choice group at P < 0.01 (Table 4-3).

Distance Score to Incorrect Response

Here also, variance homogeneity permitted a parametric one-way analysis of variance. This time the differences in mean score were significant at the 1% level and the STP test showed that these differences were concentrated between the squeak-control and choice groups and the click-control and choice groups. There was no significant difference between the two control groups (Table 4-3).

A T-test on the differences in mean scores to the correct side and to the incorrect side for each group showed that all three groups differed significantly in their distance scores (squeak-control = P < 0.05, clickcontrol = P < 0.025, choice = P < 0.001) although the choice group differed the most. The click-control group went farthest to the incorrect side while the squeak-control and the choice groups went farthest to the correct side, i.e., to the recorded squeaks.

Number of Choice Reversals

The number of choice reversals was defined as being the number of times a mouse triggered a photocell on the opposite side of the SCB from the previous mark in any one trial. A mean number of reversals was calculated for each mouse. The variances in this measure were non-homogeneous so the Kruskal-Wallis one-way analysis of variance


was used to test for a difference in means. The results of this test showed that the mean number of reversals did differ between the groups (0.02 > P > 0.01). The click-control group ran back and forth the most and the choice group the least (Table 4-3).

Discussion:

The results of this test of distress squeaks as a factor in activating mother mice to search were interpreted as supporting the hypothesis that the squeaks do cause the mother mice to come out of their nests to search for the source of the sound. Since the only cues present were the recorded squeaks there was no possibility that other channels of communication were available to carry this information to the mouse. Had live pups been present with their unique combination of odors, sounds, and tactile sensations the retrieving responses of the mothers might have been faster and even better directed, but with sound alone the mice went as close to the sound source as they could get and struggled to get closer.

There can be no question that the mother mice readily distinguished the recorded squeaks from the "white" noise. They showed by their differential approach behavior that they preferred the squeaks. There did not appear to be any aversive factor to the "white" noise as the mice readily entered the arenas with the "white" noise when they continued their searches for the invisible squeaking pups. The mice



would have meant going to the clicking side, and went less far to the clicks. The mice appeared to have no trouble differentiating the three sounds.



CHAPTER 5

Species-specific Discrimination Based on Auditory Cues: Discrimination Test.

<u>Peromyscus maniculatus</u> females remained in their nests and did not respond to recorded distress squeaks of <u>P.californicus</u> pups in a pilot study (personal observations, 1968), although in the same test they did respond to the calls of pups of their own species. This suggested that some species-specific discrimination of distress calls was possible and that comparisons of the responses of mice of different species to recorded distress calls of each species might elucidate the ability of mice to distinguish related sounds.

If the differences between the distress calls of different species, as shown in Chapter three, are the result of character divergence, then sympatric species should not only show physical differences in their calls but should also be able to differentiate them. In our trapping areas in central lower Michigan we had trapped <u>P.maniculatus bairdi</u> and <u>P.leucopus</u> in the same trap line. Whether this shows territorial overlap as well as geographical sympatry is not clear but the mice were surely within hearing range of each others' squeaks. For the maintenance of separate niches and efficient propagation of the species it would be



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advantageous for these mice to have some means of recognizing conspecifics at a distance.

I therefore hypothesized that if there was no conspecific recognition of infant distress calls, mother mice would be unable to demonstrate a choice between calls recorded from con-specific and non-specific pups and played in the SCB. As it was evident from observations in the colony that <u>P.leucopus</u> mice tended to be more "nervous" and generally active than the <u>P.maniculatus</u>, I decided to test similar choice and sound-control groups of both species. Differential discriminatory abilities were not unexpected although discrimination due to increased activity could not be separated from discrimination due to superior hearing.

Subjects:

Twenty-four lactating <u>P.maniculatus bairdi</u>, and 16 lactating <u>P.leucopus</u> were used in this test. Each species provided two experimental groups to which mothers were assigned, in sequential order, as their litters were born. Maintenance and pretest procedures were identical to those in the Activation test. The 24 <u>P.maniculatus</u> were all F_1 daughters of wild-caught parents. Four of the <u>P.leucopus</u> had been wild-caught themselves as adults and the rest were first to third generation offspring of wild-caught mice. Earlier studies on mice from the same stocks had shown that even 17 generations of semi-domestication in the laboratory did not significantly alter activity in a novel environment



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ч. С in the squeak-control group searched for the source of mouse squeaks and went correctly to the source a significant number of times.

Mice in the click-control groups did not hear the mouse squeaks but rather sounds only superficially representing them. In this case their correct responses did not significantly differ from random, i.e., that which would have been expected if they had just been alternating sides. The sounds did activate them to leave their nests. But they neither came out so much, nor so fast as the groups hearing mouse squeaks. When distances were calculated this group was seen to have gone farther to the negative side, that is, the "white" noise, suggesting an avoidance of the clicks.

When they were given a choice between squeaks and clicks all of the mice preferred the squeaks. Although intermediate response latencies suggested that the mice in the choice group actually had to chose sides, when they came out they significantly chose the squeaks. Once in the arena with the squeaks they tended to go farther, and more of them went all the way to the speaker where they tended to stay, leaping at the walls, continually triggering the photocell, as if attempting to get closer to the source. In part, the long time spent at the squeaking speaker explains the greatly reduced choice reversals in this group. The results from the click-control group suggest that the mice in the choice group had fewer choice reversals, which



(Price, 1967). Although a correlation between semi-domestication and latencies to enter an open field was found in that study (Price, 1967) only three generations of laboratory conditions were not expected to have any discernable difference. In addition some of the <u>P.maniculatus</u> had been used in the Activation or Localization tests, or both, but as the object was to demonstrate species differentiation a certain practice in retrieving or additional familiarity with the test box was not expected to affect the test results.

Equipment:

All of the apparatus used in this test was identical to that used in the Activation test. The sounds played to the mice differed, in that the recorded squeak of a 7-day old P.leucopus was substituted for the clicks.

Procedure:

In order to allow comparisons between the groups in both the Activation test and this Discrimination test, testing protocols, with one exception, and all pretest procedures were the same in both tests (see Procedure - Chapter 4). For each species the squeak-control group heard the <u>P.leuco-</u> <u>pus</u> squeaks versus white noise and the choice groups heard the recorded squeaks of the two species simultaneously (Table 5-1).



clicks clicks "white" noise "white" noise "white" noise "white" noise groups in the Activation and Discrimination tests. Each group of mice heard two sounds simultaneously from opposing speakers Sound Stimuli leucopus leucopus leucopus DISCRIMINATION TEST ACTIVATION TEST maniculatus maniculatus maniculatus in the Sound Choice Box. я 14 14 12 12 14 ∞ squeak-control squeak-control squeak-control click-control Group choice choice P.maniculatus P.maniculatus Species P.leucopus

leucopus

maniculatus

ω

choice

Table 5-1

Comparative protocol of stimulus presentations to experimental



Variables:

Since this test was an outgrowth of the Activation test the independent variable was again the sound presented. Other factors which might have influenced the response of the mouse, such as age, sex, experience, reproductive state, age of own litter, and some attributes of the stimulus were also held constant. The dependent variables of latency-toenter, choice, and distance were recorded by the event-recorder and analysed. For the squeak-control groups a positive response was considered to be a response to the <u>P.leuco-</u> <u>pus</u> squeaks and for the choice groups a positive response was one to the con-specific squeaks.

Results:

As in the Activation test the dependent variables were considered to fall into the two categories of those related to general activation and those related to preference. Response latencies and total numbers of responses were considered to be measures of activation and numbers of positive responses, reversals, and distances traveled were measures of preference.

Litter Age

Over all the groups in this test the mean litter age was 7.6 days, slightly older than the litters of the mothers in the Activation test (Table 5-2). Within the four groups of the Discrimination test the mean litter age did not differ significantly (Kruskal-Wallis one-way analysis of variance)



Table 5-2

Measures of homogeneity of reproductive state and responsiveness of <u>Peromyscus maniculatus</u> and <u>P</u>. <u>leucopus</u> mothers in four experimental groups of the Discrimination test: Litter age (days) and Latency-to-calm (seconds).

		Litte	er age	Latency-to	o-calm
Groups	n	x	S.E.	$\overline{\mathbf{x}}$	S.E.
P.maniculatus					
squeak-control	12	8.2 +	0.32	194.5 <u>+</u>	43.58*
choice	12	7.6 <u>+</u>	0.38	135.5 <u>+</u>	47.77
P.leucopus					
squeak-control	8	7.9 <u>+</u>	0.50	410.5 <u>+</u>	95.67
choice	8	6.8 +	0.78	222.9 +	73.76

*n's reduced in all groups in calculation of mean and standard errors due to missing data.



and I thus considered them to be at essentially the same point in their hormone cycles.

Latency-to-calm

Over all four groups the mean time for the mothers to calm down after the removal of their pups, until they had remained in their nests for 30 seconds did not differ significantly (Kruskal-Wallis) one way analysis of variance). In the Activation test the latency-to-calm was measured at the end of one minute but in this test the <u>P.leucopus</u> mice were found to be so active that they were rarely still for one minute after their pups were removed. In order to have a standard of comparison between the two species the waiting period was therefore shortened to 30 seconds (Table 5-2). Total Number of Responses

All of the <u>P.maniculatus</u> mice responded in every trial, thus reducing the over-all variance sufficiently to permit a parametric analysis of variance. The one-way analysis of variance demonstrated that the groups differed significantly in mean number of trials in which there was at least one response (P < 0.005). This significance was attributed to the greater number of trials in which the <u>P</u>. <u>maniculatus</u> groups and the <u>P.leucopus</u> squeak-control groups responded than the <u>P.leucopus</u> choice group (New Multiple Range Test; <u>P.maniculatus</u> groups differed from <u>P.leucopus</u> choice P < 0.01; <u>P.leucopus</u> control differed from <u>P.leucopus</u> choice P < 0.05; Table 5-3). The greater response of the



Table 5-3

Mean number of responses and mean response latencies of <u>Peromyscus</u> <u>maniculatus</u> and <u>P.leucopus</u> mothers to distress squeaks of pups of both species <u>and control</u> sounds in the Disscrimination test.

	P.maniculatu	IS	P. leucopu	ω	
	Squeak-control	Choice	Squeak-control	Choice	д ,
п	12	12	ω	8	
Trials with a response	4.00	4.00	3.88	3.50	0.005
Number first responses correct	3.17	2.00	3.00	2.25	n.s.
Latency to first response (seconds)	66.24	35.40	55.09	142.24	n.s.
Latency to correct response (seconds)	80.55	70.87	90.47	121.79	n.s.



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<u>P.maniculatus</u> mice was possibly related to the prior experience of some of them in the SCB, as well as to a genuine species difference in excitability.

Number of First Responses Correct

The mean numbers of first responses to the "correct" side of the SCB, i.e., to the squeaks for the squeak-control groups and to the conspecific squeaks for the choice groups, did not differ significantly when analysed with a Kruskal-Wallis non-parametric analysis of variance (Table 5-3).

Considering total initial responses for all the mice in each group, all the <u>P.maniculatus</u> mice responded in all trials. The two squeak-control groups (<u>Pleucopus</u> & <u>P.maniculatus</u>) responded non-randomly, that is, chose squeaks more than noise, 79.2 & 78.2 percent of the time, respectively, significant at 0.001. In contrast, the <u>P.maniculatus</u> choice group did not respond in a manner distinguishable from random, being correct only 50% of the time. The <u>P.leucopus</u> choice group differs from the random expected percentage correct (50%) at the 0.05 level.

Latency to First Response

As in the Activation test this latency was measured as being the time between the onset of the test sound and the first recorded response of the mouse. These latencies showed a heterogeneous variance which required the use of the non-parametric Kruskal-Wallis one-way analysis of variance. The results showed no differences in mean latency to respond (Table 5-3).



Latency to Correct Response

The time for a mouse to respond to the "correct" stimulus did not differ significantly between the four groups. Variances were large and again heterogeneous so the Kruskal-Wallis test was used (Table 5-4).

Distance

Defined, as in the Activation test, as the distance a mouse moved in either arena towards a stimulus sound, this variable was measured in terms of scores of photocells triggered. A mouse that ran all the way to the speaker in each trial would accumulate a mean score of 3 while movements into the tunnels only would accumulate a mean score of 1. For additional comparisons between the two species the differences between correct and incorrect scores for each group were calculated (Table 5-4).

Distance Score to Correct Response

These scores were found to show heterogeneous variances requiring the Kruskal-Wallis one-way analysis of variance. The means were not found to differ between the groups (Table 5-4).

Distance Score to Incorrect Response

Since these data showed homogeneous variances a parametric one-way analysis of variance was possible. This test showed significant differences between the groups (P < 0.05). Individual groups were then paired and tested with the New Multiple Range test showing that the P.maniculatus squeak-control and choice groups differed at P < 0.01,

Table 5-4

Mean distance scores and mean choice reversals of Peromyscus maniculatus and P.leucopus sponses and for choice reversals. With non-parametric (Kruskal-Mallis) and parametric analyses of variance. Student's t-test used to calculate significance of differences mothers responding to distress squeaks of pups of both species and control sounds. Differences between groups computed for distance scores of correct and incorrect rebetween distances traveled to correct and incorrect stimuli.

	P.maniculat	SI	Sunnaniel d		
	Squeak-control	Choice	Squeak-control	Choice	д
ц	12	12	8	8	
Distance correct response	2.81	2.42	2.44	2.00	n.s.
Distance incorrect response	0.85	1.71	1.13	1.03	0.05
Choice reversals	3.00	5.42	2.63	2.88	n.s.
Difference between correct and in- correct score	1.96***	0.71**	1.31*	0.97**	
*** P .005					

** P .01



with the mice in the choice groups going farther to the incorrect sound (<u>leucopus</u> squeaks), than the squeak-control group. The <u>P.leucopus</u> groups did not differ on this measure. But the <u>P.maniculatus</u> choice group differed from the <u>P</u>. leucopus choice group at P < 0.05.

A Student's t-test on the differences of the paired scores to the correct side and to the incorrect side for each group showed that all mice went significantly further to the speaker producing the squeaks of their own species (or any squeaks for <u>P.maniculatus</u> squeak-control group) than to the opposite side.

Number of Choice Reversals

The variances in the mean number of choice reversals were homogeneous so a parametric one-way analysis of variance was run. The differences in these numbers were not found to be significant over all four groups (Table 5-5).

Discussion:

The results of this test confirm the findings in the Activation test that deermouse mothers search for the source of infant distress squeaks. Here, a second species, <u>P.leucopus</u>, showed increased activity and left the nest when distress calls were played. These mice approached mouse squeaks more frequently, and closer, than the "white" noise. In the choice test, with recorded squeaks from a 6-day old <u>P.maniculatus</u> and a 7-day old <u>P.leucopus</u>, the <u>P.maniculatus</u> mothers responded randomly on all but one



variable. One measure, the comparison between the distance score to the correct and incorrect stimuli, showed a significant difference from random. This difference in scores was smaller for the choice group than for the squeak-control group but it was significant. The <u>P.maniculatus</u> mothers went closer to the <u>P.maniculatus</u> squeaks than to the <u>P.</u> leucopus squeaks when they had a choice (Figure 5-1).

The <u>P.leucopus</u> mothers also showed a significant difference in distances traveled to the conspecific versus the non-specific sounds. In addition the <u>P.leucopus</u> choice group showed a higher percentage of correct responses to the conspecific sounds.

These data are interpreted to mean that while it appears to be possible for deermice (at least of these two species) to differentiate distress calls of pups of their own species from others, the recognition is difficult and the sounds are perceptibly similar. It may not be necessary to differentiate the calls in the natural situation if the mice live in different niches and rarely meet.

Bradshaw (1965) and Moore (1965) have shown that adult mice of these species can differentiate conspecifics from mice of other species on the basis of odor alone, so it is probable that the adults could recognize pups of their own species by odor. If the pups can be recognized as appropriate to retrieve when the mother arrives, it may not be necessary for her to be able to differentiate before she leaves her nest. It may be that even with



Figure 5-1 Legend

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Distance score to sound sources in Activation and Discrimination tests. Photocells recording mouse activity were numbered 1, 2, or 3 depending on their distance from the nest/start box in the SCB. Mean scores reflected the distance traveled by the mouse towards a particular sound source as averaged for each mouse over four trials. Scores towards the stimulus defined as "positive" above the abcissa, those defined as "negative" below the abcissa. P values for differences between "positive" and "negative" scores printed on top of each bar.





sympatric species the situation rarely arises where the distress cries of a pup of one species are overheard by a mother of the other species. Differences in the squeak recognizable by the mice may be due to physical differences in the mouth and vocal cords and not the result of character divergence. The test described here merely demonstrates a perceptible difference but cannot clarify the cause of the difference.

Comparison of Results of Activation and Discrimination Tests:

The results of these two tests can be compared in terms of the responses of <u>P.maniculatus</u> mice to squeaks of pups of their own and the other species and in terms of differences in the behavioral responses of the adults of both species to similar stimuli in similar situations. Although the experimental groups were unequal in size, the experimental apparatus and protocols were identical and so I felt that non-parametric statistical comparisons of the results were justified.

When the retrieving responses of the mice were considered within species but across experimental groups the two species demonstrated similar trends. Both <u>P.manicula</u>tus and <u>P.leucopus</u> mice chose squeaks of pups of their own species over "white" noise. They made more correct choices when they were offered squeaks versus the control sound than when they had to choose between two squeaks. Distance scores


showed that both species went farthest towards the conspecific squeaks when presented with the choice situation (Figure 5-1).

<u>P.maniculatus</u> and <u>P.leucopus</u> also demonstrated a preference for conspecific squeaks in terms of total responses (Figure 5-2, Table 5-5). Again both species showed strong preferences for squeaks of either type over the control sounds. Response latencies, however, were longer for <u>P.leucopus</u> in the choice trials than for <u>P.maniculatus</u> (Figure 5-3). A certain portion of this difference can be ascribed to the greater familiarity of the <u>P.maniculatus</u> mice with the apparatus since their earlier latencies (in the Activation test) were comparable to the <u>P.leucopus</u> latencies.

Choice reversals reflect the difficulty of the choice between the squeaks of the two species, even for the more experienced <u>P.maniculatus</u>. Reversals are significantly greater for the squeak choice groups (Figure 5-4).

These comparisons support the conclusions already reached from the Discrimination test results - i.e. that while it may be possible for <u>Peromyscus</u> to differentiate pups of closely related species on the basis of sound alone, they do not always do it. The cues which make the sounds acceptable to the females of the different species may differ simply from anatomical differences due to physical divergences rather than from specific character divergence of the sound characteristics. More widely

Figure 5-2

Legend

Mean numbers of responses to the sound sources, averaged for each mouse over four trials in the Activation and Discrimination tests. Responses to "positive" sounds above the abcissa, those to "negative" sounds below abcissa. Random response levels marked with arrows at the level of 50% total response for each side.



FIGURE 5-2



Table 5-5

Comparison of the responses of <u>P.maniculatus</u> and <u>P.leucopus</u> mothers to squeaks of <u>con-specific</u> pups In control situations (squeaks versus "white" noise) and in choice situations (con-specific squeaks versus squeaks of non-specifics). Numbers of mice responding more than 50% of the time.

	con-specific squeaks versus "white" noise			con- squea non-spec	spec ks ve ific	pecific s versus fic squeaks	
	obs.	exp.		obs.		exp.	
P.maniculatus							
n-14	12	7	n=12	2		6	
P.leucopus							
n=8	6	4	n=8	4		4	

Chi-square 0.01 > P > 0.005



Figure 5-3

Legend

Mean response latencies in the Activation and Discrimination tests. White bars are latencies to any (first) response. Dark bars are latencies for responses to the "positive" (correct) sound stimulus. Mean values for latencies in seconds marked at the top of each bar.



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FIGURE 5-3



Figure 5-4 Legend

Mean choice reversals in the Activation and Discrimination tests. Bars indicate mean number of times mice ran from one arena through the nest/start box and entered the other arena. As the entire length of the SCB could be traversed by a running mouse in less than a second the distance of each entry into an arena is not computed here. P values for significance of the differences between groups were P < 0.05 for the maniculatus squeakcontrol,click-control and maniculatus-squeak/click choice groups and P > 0.05 for the four leucoupus squeak groups.





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divergent species would be expected to reject sounds of the other species more conclusively.



CHAPTER 6

Complete and Attenuated Distress Cries as Sound Targets: Localization Test.

Since both a squeaking pup and a searching mother mouse expend energy and risk alerting predators, it is important that the mother find the pup as fast as possible. Short, sharp, high-frequency sounds, such as these mouse squeaks, are theoretically easy to localize with binaural sound reception devices, i.e. a pair of ears (Marler, 1967). Observations on wild mice indicate that pups dropped by a mother fleeing a disturbed nest are quickly located and retrieved. Obstacles and darkness do not appear to affect the accuracy of the search.

I hypothesized that neither visual nor olfactory cues are necessary for localization of a squeaking pup but that sounds provide sufficient and essential information for a directed search. Sound sources providing only audible cues should be located as precisely as sources with the additional visual and olfactory information from living pups. In nature while odors may be limited in effectiveness as signals at any distance, particularly downwind, sounds should carry well in any direction from a point source. Using any or all of the possible methods of





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localizing sounds (discussed in Chapter 3) a mouse should be better able to follow a sound to its source than an odor, even in dark or obstructed habitats where sight is impossible.

Accordingly I designed a box which permitted alternative presentation of sounds devoid of accompanying visual or olfactory cues (recorded squeaks) and sounds with all the additional cues of a living pup except visible ones (live pups behind a light-opaque, sound-transparent barrier). The box was semicircular with alleys fanning out from the center of the circle so that sounds could be presented at eight positions equidistant from the subject. The alleys helped to channel the sound and force the mouse to chose a direction while still at a distance from the sound source.

Subjects:

The test animals comprised one group of 42 lactating <u>P.maniculatus bairdi</u>. Four of these animals were wildcaught as adults, and 38 were F_1 daughters of wild-caught mice. The scores of the two groups did not differ significantly on any measure so the results were lumped into one group. All of the mice were nursing litters of 5-10 day old pups (\bar{x} age of litter = 7.4 days). The F_1 mice had almost all been used in either the Activation or the Discrimination tests or in both, but as their scores did not differ significantly from the naive mice, experience



at retrieving in another apparatus did not seem to produce inhibitions. As the point of the test was to test a maximum accuracy in locating a pup and not ability to retrieve, experience was not judged to be a negative factor.

Equipment:

Test squeaks were recorded in the same manner as for all the other tests, using the same equipment and they were played back to the mice in the same way. The experimenter stayed in the room with the mouse in order to visually observe her behavior. Tape recorder and amplifier remained in an outer room and were operated by remote control, leaving the speaker and remote control switch as the only equipment in the room. Illumination was supplied by one 10-watt,red-light bulb hanging over the center of the circle formed by the Fan box. This provided just barely enough light for the observer to see the movements of the mice and there were no shadows formed by the sides of the alleys. Despite the light and the presence of the observer the mice left the start cage and readily searched in the Fan box when there were squeaks.

The Fan box (figure 6-1) was designed to make each sound source equidistant from the exit of the start cage. The outer edge of the box circumscribed a portion of a circle having an arc of 120° and radia which, if continued into the center, formed angles of 15°. The Fan box was divided into eight wedge-shaped compartments with the small



Figure 6-1 Legend

Fan Box: A mouse placed in the cage at the center of the fan is exposed to sounds emanating from a speaker (S) placed at the end of any alley (A). Perforated barriers (B) prevent the mouse from seeing either the speaker or a live pup placed at the end of the alley but do not restrict odors and sounds. Walls of the alleys form angles of 15° at the center. Complete outer walls are 36 in. long, and inner alley walls are 18 in. long.





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end of the wedge (4-2/3") open to the center and the large end (9-1/3") at the edge of the circle. A rectangular opening (6" X 5" high) cut into the plywood outer ends of these wedges and covered with screen and black fabric, provided a sound-transparent shield for the speaker outside the box. The plywood sides of the wedges extended into the box radially for 18", half-way to the center. Each wedge thus formed an alley down which the mouse could approach the source of the sound. Three inches from the wide outer end of each wedge a perforated barrier (8-1/2" X 2" high) prevented the mouse from seeing the pups used as live sound sources. The outer walls of alleys number 1 and 8 continued to the center of the box and joined the entrance to the start cage. This cage was a standard colony cage modified so as to have a door (2" X 3" high) which could be raised from above to let the subject into the Fan box. Except for the start cage the entire Fan box was painted flat black.

Procedure:

Each mouse was put into one of the modified cages with her litter, food, water, and clean bedding, at least 48 hours prior to the test. On the day preceding the test the mouse was allowed to explore the Fan box for 30 minutes. During this time her litter was randomly placed in the box to encourage her to enter and retrieve them.

At the time of the test the mouse's cage was



positioned at the opening to the Fan box, her litter was removed to another room, and she was allowed to calm down for five minutes. Then the first stimulus was presented, either a live pup of the mouse's own or the recorded squeaks of a 6-day old pup. The stimuli were initially presented from a randomly chosen alley; additional presentations were made in similar fashion except that no more than one presentation of each type was made from any alley. Alleys number 1 and 8 (at the far right and left) were never used, in order to avoid a strong bias due to the tendency of the mice to follow along the walls of the box.

Sounds were presented for 30 seconds before the door of the start cage was raised letting the mouse into the box. The sound continued for five minutes or until the mouse had made three choices, whichever came first. A choice was defined as an excursion past the halfway mark in any alley. After three choices, correct or incorrect, the sound was turned off and the mouse returned to the cage. The next sound was presented after a one minute waiting period. If a mouse did not respond in five minutes it was scored as zero response and 5 minute latency. Mice with three consecutive zero scores were replaced as subjects.

As stimuli, recorded squeaks alternated with live pups. Each subject was given six trials, three with each stimulus. As each thus served as her own control, her responses to live pups and recorded squeaks could be



compared. Almost all of the mice retrieved the live pups and these pups were allowed to remain in the nests during subsequent trials with no apparent effect on the mother's behavior. Unused pups were returned to their mothers at the end of the tests.

In this experiment the independent variable was the nature of the stimulus, whether live pup or recorded sound. The dependent variables were the correctness of the choices of the mice, their response latency, and the numbers of responses. Since each mouse served as her own control, there were, in effect, two paired experimental groups which could be compared in terms of responses to live pups or recordings.

Results:

All but 8 of the mice responded in all trials. Four of the mice which refused did so in 2 consecutive trials; four mice refused to respond in only one trial. These null trials (no response) were equally divided between live stimuli and recordings. Whether the mouse's first trial stimulus was live or recorded made no significant difference in the number of responses. Mice which refused to enter the box often spent the trial time flipping in their cages, a behavior which was interpreted as an indication of a conflict between fear of the apparatus and the impulse to search for the squeaking pup. When the door was lowered and the sound turned off at the end



of the trial, the mouse always stopped flipping. Latencies

Latency to enter the Fan box after the door was opened and the latency to arrive (touch the barrier) at the end of the alley were separately measured. Mean latency to enter for recorded sound was 35.78 seconds and 38.51 seconds for live pups (Table 6-1). Since each mouse served as her own control the Wilcoxon non-parametric test was run on paired data but the differences in entrance latencies for the two stimuli were not found to be significant.

On the average it took a mouse 15.15 seconds for a mouse to reach the end of an alley after it entered the box on hearing the cry of the live pup, and 25.38 seconds when it heard the recorded squeaks. Arrival latencies, calculated as the time elapsed from the opening of the door until the mouse arrived at the distal end of the alley, differed between the stimuli (Wilcoxon, P = 0.05, Table 6-1). Mean arrival latencies were 53.66 seconds to live and 61.16 seconds to recorded sounds.

Choices

Predictions of the behavior of the test animals in the box were based on the number of alleys and the number of positions where the mouse could end up if it behaved in an undirected manner. Although sounds were only presented in 6 alleys the mice were free to enter all 8 alleys. Any mouse responding to the stimulus might be correct, or wrong



Table 6-1



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("off") by one alley (15°), 2 alleys (30°), 3 alleys (45°), 4 alleys (60°), 5 alleys (75°) or 6 alleys (90°). Predictions were complicated by the fact that since the box was only semi-circular, not all choices were open for all of the 6 possible positions of the speaker. If the stimulus came from alley number 4, for example, then a mouse could only be off by 4 alleys on the right and 3 on the left.

Probabilities of a mouse being correct or wrong by a particular number of alleys (degrees) were calculated (Table 6-2). Expected numbers of responses and percentages were compared with observed responses with the Chisquare test. Predictions of random behavior, unrelated to the source of the sound, produced the ratios in the expected columns. The observed responses were found to be highly significantly different and overwhelmingly oriented to the source of the sound (Table 6-2).

If only the first response of the three permitted in any one trial was considered there was a significant trend to more correct orientations when the stimulus was the live pups than when it was recorded squeaks. However if the best choice of the three was considered there was no significant difference between responses to recorded and live squeaks.

First choices are schematically represented in Figure 6-2, where the arrows correspond to the percentages of the total responses to correct and other alleys when the stimulus was in various positions. Solid arrows


three trials. Correct choice refers to a choice of the alley containing the sound source. Alley choices of Peromyscus maniculatus mothers in Fan box, searching for source of live or recorded infant distress cries. Random expected choice compared to observed with Chi-First choice in a trial and best choice of three in a trial arranged over One off, two off, etc., refer to adjacent alleys. square test.

irst choice	N = 42	Average Nu	umbers of N	dice			
	expected		observed]	Live sound	observed	recorded sound	
Choice	×	qip	IX	qij	ıx	ор	
correct	5.25	12.5	21.3	50.7	14.0	33.3	
L off	10.50	25.0	8.7	20.7	11.3	26.9	
2 off	8.74	20.8	3.3	7.9	6.7	16.0	
3 off	7.01	16.7	3.0	7.1	3.3	7.9	
1 or more	10.6	25.0	3.3	7.9	4.7	11.3	
no choice	0	0	2.0	4.8	2.0	4.8	
Observed v	s. Expected	Chi-square	P < < 0.0	100	0.01 > P	<pre>> 0.001</pre>	
Best Choice							
	expected		observed]	Live sound	observed	recorded sound	
Choice	IX	сір	IX	dР	ıx	dР	
correct	5.25	12.5	34.0	81.0	26.7	63.6	
L off	10.50	25.0	3.7	8.8	7.7	18.3	
2 off	8.74	20.8	1.0	2.4	2.0	4.8	
3 off	1.01	16.7	1.0	2.5	0.7	1.7	
t or more	10.50	25.0	0	0	0.9	2.1	
no choice	0	0	2.0	4.8	2.0	4.8	
Observed vs	s. Expected	Chi-square	P < < 0.0	100	P < < 0.	100	

Table 6-2



represent responses to live squeaks and broken arrows responses to recorded squeaks. The diagrams dramatize the accuracy with which the mice directed themselves to the sound source. Had best choice of three been diagramed the picture would have been even more clear cut.

Although the design of the experiment made it rare for a stimulus to be presented twice for the same alley, in one test, on those occasions when it did happen there was no observational evidence that the mice returned to the site of a successful retrieve rather than searching for the source of the sound. There was, however, a tendency for the mice to follow along the walls of the box and then cross the opening to the alleys, listening at each one. At the rapid rate at which they moved this behavior often caused them to mistakenly enter the alley preceding the correct one. Those mice which were correct most consistently, tended instead to linger at the entrance to the box, turning their ears until they appeared to get some sort of fix on the sound and then went directly to the end of the appropriate alley.

Discussion:

The results of the analyses of the choices of the mice responding to both live and recorded squeaks indicate that the sound alone is sufficient to provide cues for accurate location at the distance of 3 feet within 15°, in this apparatus. While first choices were better with



Figure 6-2

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Legend

Diagrams of relative percentages of responses in correct and incorrect directions by Peromyscus maniculatus mothers searching for sounds of live and recorded distress squeaks. Live squeaks as stimuli - sold arrows; recorded squeaks - broken arrows. Arrows represent percentages of responses, not relative distance since all responses recorded went the minimum distance. S marks placement of the sound source. Multiple diagrams are presented as the positions of the sound source are logically independent and are paired on the page so as to show right and left sides of the Fan box. Notice increased edge effect, especially with recorded sound when the source is in allevs 2 and 7 (diagrams e & f). Long arrows indicate high percentages, short arrows lower percentages, and alleys with few or single responses are not indicated. Only the highest, not all the percentages are printed.

FIGURE 6-2



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21.1 % 21.1 % 21.1 %











live pups as the stimulus I propose that this was not due to non-sonic cues emanating from them, but rather from the fact that the sound was not attenuated by the recording process and the volume was more natural, although by no means constant. In addition the longer latency to enter for mice responding to live squeaks correlates with the observation that the mice lingered more often at the doorway when the stimulus was live than when it was recorded. Those that lingered were more accurate at finding the correct alley. When the stimulus was recorded squeaks,mice tended to dash out into the Fan box and run along the walls in a less directed, less efficient search. This behavior is reflected in the longer arrival latencies when they responded to the recorded sounds.

Certainly, when best choices are considered and even when only first choices are counted, these deermice were all highly successful in finding the "pups." When pups were actually present they could get them back to the nest, and this meant carrying them over the 2-1/2" barrier, in less than a minute from this distance. Even in a dim and obstructed natural habitat, especially one familiar to a mouse, squeaking pups could easily be located and retrieved in a minimal time.



CHAPTER 7

Ultrasonic Distress Cries and Maternal Response: A Simple Communication Paradigm

The assumptions on which this study rested were 1) that infant deermice (Peromyscus) emit sounds when removed from their nests and deprived of maternal warmth and tactile stimulation; 2) that these sounds are predominately composed of frequencies above the limits of human hearing (i.e. above 20 kHz, ultrasonic) but are susceptible to electronic analysis; and 3) that these cries play some stimulatory role in the retrieving behavior of lactating female deermice. I suspected, in addition, that the cries might vary according to phylogenetic relationships and that they would show ontogenetic changes. Reviews of the pertinent literature supported the primary assumptions and confirmed the possible existence of phylogenetic variety and ontogenetical change. This study showed that Peromyscus pups do emit ultrasonic distress cries when subjected to the mild cold stress of being out of their nests and that these sounds are sufficient to alert and direct mother mice to retrieve them. Phylogenetic and ontogenetic differences in the sounds were demonstrated.

Following the model presented in Figure 7-1, the



Figure 7-1 Legend

Model of a simple communication system, showing pathways of information transfer. In the paradigm discussed here the information source becomes the mouse pup, its vocal cords and mouth are the transmitter, the signal is a sound with a message on the state of the pup coded in the physical characteristics of the signal. The sound heard (received signal) by the mother mouse (receiver) may have been distorted by extraneous noises. The arrival of the message at its destination is demonstrated by the behavior of the receiver.



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information transfer between pup and mother would have the pup as the information source, the emitter, and the mother as the receiver. The pup transmits a message coded in an age-specific, species-specific signal, using its vocal cords and mouth as the transmitter. The signal is transmitted in the sound channel, where other sounds of laboratory or woodland may intrude as noise and distortion. If the pup repeats the signal enough (redundancy), the information will be received by the relevant receiver, the mother. When she acts on the information received then we know the message has been perceived.

The results of the experiments described here, now provide us with sufficient information to consider the functional aspects of the source, the signal, and the receiver, and place them in the communication system. We can fit the pieces together in this auditory system as has been done before for visual communication systems such as the classic stickleback mating sequence.

Why does the pup signal?

An infant rodent pup often emits ultrasonic cries when stressed by cold temperature or by being touched, bumped, or handled. The recent discussions of the physiological trigger for the onset of squeaking in rodent pups leave no doubt of the importance of low temperatures. Hart and King (1966) suggested a connection between distress cries in Peromyscus and temperature, and Smith (1972)

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presents confirming data on the close relationship between these calls and temperature. Okon (1970a, b, 1971) clearly relates distress calls in white mice, white rats, and hamsters with developing homeothermy. As pups of these species develop temperature regulation they cease squeaking. With the exception of the Hart and King study, these data were published after the completion of the experiments discussed here, but the results are in complete agreement.

The temperature in my recording apparatus was maintained at about 18° C (65° F), similar to the 20° C used by Okon, and thus constituted a condition of cold stress. Smith (1972) considers 35° to be nest temperature in Peromyscus, so my recording apparatus should have been sufficiently cold to induce the spontaneous production of distress cries to cold. Once removed from the nest the pups lay guietly in the recording funnel and were neither touched nor could they fall over during the recording session. Pain squeaks deliberately elicited by pinching tails were analysed separately. Thus handling can be discounted as a stimulus for the squeaks I recorded, even though I did not suspect the existence of separate "handling" and "cold stress" distress cries, at the time. As I was primarily concerned with the physical characteristics of the sounds I did not vary the temperature.

Preliminary observations convinced me that the rate of calling was highly variable and also dependent on the



motivation of the pup, so the number of calls in a prescribed time were not measured. I predicted that the number of squeaks in a set were probably a characteristic related to phylogenetic differences, as data in Hart and King suggested (1966). Subsequent studies (Okon, 1970a; Smith, 1972) confirmed that squeak rate was closely related to temperature and the homeothermic ability of the pup.

Similarly, intensity was not measured because the movements of the mouse's head were impossible to control without restraint, which would have introduced a handling factor, and I also felt that the intensity of squeaking was a motivational factor. Okon (1970b) and Smith (1972) also found intensity (SPL) correlated with temperature in 3 general age groups. Up to 5-6 days, squeaks of albino mice are generally weak, stronger, then, until 12-13 days and thereafter weak again.

What is the signal?

The squeaks produced by the mice I recorded are generally long, high sounds with multiple harmonics and a small amount of frequency and intensity modulation. The greatest energy is usually found in the second or fourth harmonic and the fundamental may be unheard. There is a sharp onset and a sharp offset, usually with a steady tone in the middle. Observations suggest, although definitive tests have not been made, that the sharpness of the onset and offset are achieved by the pup opening its mouth after



the start of the sound production and closing it again before the end of the squeak.

Two types of distress squeak became apparent in the phylogenetic and ontogenetic comparisons. Examples of both types are sufficiently common and clear to show that they are not experimental artifacts. Type I squeaks show one strong band of energy, usually the second harmonic. often with few, weak, or no other harmonics visible. Type II squeaks are complications of the same pattern, having equally strong energy components at a frequency and its harmonic, usually the second and fourth harmonics. It should be mentioned that these 2 types of squeak have no relationship to the 2 types of squeaks and chits described for adult Peromyscus by Houseknecht (1968). Rather, I interpret them as variations on a theme by different taxa, perhaps occasioned by differentially thick vocal cords. There may be a correlation between the size of a mouse and the tendency to produce Type II squeaks, as I recorded these squeaks mostly from the larger mice, P.californicus, P.difficilis and P.m.gracilis. The fact that these Type II squeaks have 2 strong energy bands must give them a perceptibly different sound quality from the Type I squeaks with a single band.

Published frequency averages for these species which do not take the entire spectrum into consideration give an unrealistic measure of the sounds. Species means for

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frequency averaged over all ages unrealistically simplify the sounds also. All of the groups for which I analyzed the sounds showed 3 age divisions for frequency modulation patterns, similar to the 3 age divisions later found for rate and intensity (Okon, 1970a, b; Smith, 1972). It is not clear, however, whether the frequency periods correspond to the development of homeothermy, as the rate and intensity changes apparently do (Okon, 1970a, b). Pinnae in several of the taxa, P.polionotus, P.m.gracilis, and P.m.bairdi, become unsealed from the head and erect at about the end of the "early" period, day 3. Ears and eyes generally open by the end of the "middle" period, about day 12. The raising of the pinnae and particularly the opening of the ears may provide the squeaking pup with some feedback on the sounds it is making and provide information on the basis of which the pup can modify the sounds. Whether the pup does use what it hears to modify its squeaks cannot be decided now.

What is the message?

Situation:

If Smith (1972) is correct in distinguishing the "isolation" cry from the "handling" cry on the basis of frequency, rate and intensity as well as situation, then the calls discussed here, from pups stressed by cold, could carry very specific situational information. Increasingly this call appears to be emitted solely by pups in the cold,



either out of the nest or alone in it. Species:

My data show clear differences in physical characteristics between species and even subspecies. All of the taxa I recorded differed from each of the others on at least one parameter (Table 7-1). The highest mean basic frequencies were found in P.leucopus and P.polionotus, and these mice always produced Type I squeaks. In contrast. P. californicus and P.m.gracilis almost always produced Type II squeaks with strong frequency components around 17-19 kHz. Frequency patterns, as predicted, were most similar in the most closely related mice, in general. Temporal parameters do not show the same clear relationships. Temporal patterns may shift more easily with physical changes in activity and body type associated with divergence in habitat usage in allopatric groups. The physical differences existing between the squeaks of mice of different species and even subspecies could provide sufficient information for species identification.

Age:

Squeaks become shorter and less frequent as pups mature, harmonics fade out and pattern variations are reduced. It is important to note that temporal and frequency characteristics do not change continuously but rather in bursts at about day 3 and days 10-12. Some gradual trends are visible in set duration and the number of squeaks in a set



Ran	ige	Clostst Relationship	Number Squeaks Per Set	Set Duration	Squeak Duration	Over- tones	Basic Frequency	Squeak Type
<u>/P.m.gracilis</u> S		species	* *	*			n.s.	same ⁱ .
/P. leucopus S/	Α,	sub-genus	*	*	n.s.		*	differ
/ <u>P.polionotus</u> A	_	species-group	n.s.	*	* *		* *	differ
<u>is/P.leucopus</u> S/,	A'	sub-genus	*	*	n.s.		*	differ
<u>is/P.polionotus</u> A		species-group	*	*	*		* *	differ
.us/P.leucopus S	10	sub-genus	*	n.s.	* *		*	same

* significant difference, P 0.05

** highly significant difference, P 0.01

n.s. no significant difference

i. P.m.bairdi produces both Type I and Type II squeaks although the Type II are the most frequent.

Table 7-1



(Figures 3-15, 3-16). No particular parameter varies consistenly across all ages or all species. It seems likely, therefore, that the listening mouse may recognize a familiar sound <u>Gestalt</u>, instead of a particular frequency or number of squeaks. Combinations of harmonics and energy investments in different frequency bands would make perceptibly different pitches. Repetition of sets may simply add redundancy, insurance against information loss due to noise or distortion.

Localization:

The cues necessary for accurate localization of sounds are all present in these distress squeaks. Marler (1961, 1967) considers that sounds can be localized by means of differences in intensity, phase, and time of arrival of the sound at paired sound receptors, ears. Localization by means of intensity differences is most effective when the sound shadow of the head prevents sound from reaching both ears simultaneously, which occurs when the wavelength of the sound is shorter than the width of the head. Data in Chapter 3 (Table 3-5) suggest that the mice in this study would only be able to use this method if fundamentals were heard. However, localization by means of phase differentials is best when the sound frequency is longer in wavelength than the width of the head. Most of the basic frequencies measured in this study are longer in wavelength than the distance between the ears of their producers so that phasedifferential localization is probable. Sharp onsets and



offsets and transients are effective in localization by means of time differences. These squeaks are composed of many discontinuities and the short, sharp squeaks are repeated rapidly in sets, making localization by means of time also probable.

In contrast, the pain squeaks analyzed had gradual onsets and offsets, many blurred frequencies and extremely fast repetition. These characteristics suggest to me that there is no need for the pain squeaks to be easily localized and may even be some advantage in disguising their source.

The analyses indicate that the distress squeak of infant rodents is far more complex, physically, and capable of carrying more information, than has previously been supposed. The squeaks are characteristically easy to locate and significantly different from species to species and for major groups of ages (or developmental stages). It is not even unreasonable to suspect a certain amount of individual variation, given the high variance noted within species, and it might be possible to demonstrate individual recognition on the basis of sound. These squeaks are undoubtedly the "audible" cries described in Smith (1972).

Noise and Distortion:

High frequency sounds are an excellent medium for information transfer in a dark, obscured habitat. They require no other energy for transmission than that invested in them at emission, they can go around obstacles, and do



not linger to give directions to predators. Short, sharp, high sounds probably encounter few other natural noises which would interfere with them and within a very short time period can transmit a large amount of information.

Other receivers besides the relevant one, the mother mouse, can of course, pick up the communication, but that is no worse a problem with sound than with any other channel. In this case, some predators cannot receive the communication; owls do not appear to hear sounds this high, and snakes cannot hear at all.

What does the receiver do?

Listening mother <u>Peromyscus</u> are stimulated to emerge from secure nests, search for, and retrieve calling pups. The results of the Activity and Discrimination tests confirm studies with other rodents and establish the importance and efficacy of these sounds in initiating retrieving behavior in at least 2 species of deermice. Mother <u>P.maniculatus bairdi</u> and <u>P.leucopus</u> overwhelmingly responded to recorded sounds of calling pups of both species. Very few responses were given to control sounds and no responses were observed when there were no squeaks.

These mice also responded in a manner suggesting discriminatory abilities. <u>P.m.bairdi</u> showed a preference on 1 measure while <u>P.leucopus</u> discriminated on 2 measures. Conspecific identification would have been expected to have been even more clear had species with more strongly divergent



sounds been used. <u>P.leucopus</u> and <u>P.maniculatus</u> were chosen in the first place for this test because they are essentially allopatric in Michigan. If there is any adaptive value in a mouse being able to differentiate pups of its own species by sound alone, it was expected to show most strongly where chance encounters between species are possible, with allopatric species. The results suggest that the physical differences in the sounds may be discernable to relevant receivers, mother mice and that they may act preferentially.

Location of the source of distress squeaks by retrieving mothers was immediate and highly accurate (Chapter 6). Squeaks were located, as well as live pups which presented the additional cues of odor and visible form. Results are conclusive enough in this test to state that sound alone is a sufficient cue for localization. Odor may be utilized on contact, for family identification, but this suggestion has not been explored.

The equipment used in presenting the recorded squeaks as stimuli attenuated the very high frequencies and undoubtedly introduced additional noise. It also presented the sounds at an unnatural sound pressure level. Nevertheless the mice emerged to search and found the sound sources with little difference in performance from the trials where live pups were used. This indicates that the frequencies which carry the location information, at least, are those middle


ones (20-40 kHz) which have the greatest energy in the squeak.

While a specific frequency may carry the location information to the listening mother, the combination of all the other characteristics of the sound, its <u>Gestalt</u>, probably informs her of the age and species of the caller. It is either recognizable as a familiar sound (conspecific) or tone variations which may be heard constantly from her own litter. As the pups in the litter develop, their sounds change, but as the mother must hear them constantly her sound search image, or recognition image, must change also. As the mother's physiological maternal motivations diminish, the pups, developing their own temperature regulation and abilities to move, squeak less and less loudly. Consequently retrieving responses diminish.

The meaning of the message?

Meaning, that is, the information in the context in which it is emitted and received, can now be better understood (Smith, 1965). Figure 7-2 is a diagram of the information system of Figure 7-1 with the behavior of the mice superimposed on it. The information of situation (isolated pup), species (conspecific), age (same as the listener's pups), and location, is contained in the squeak that the female hears. When she emerges and retrieves the pup, the information transfer is complete, the message has been received, the meaning comprehended.



Figure 7-2 Legend

Representation of distress squeak communication system. After nest disturbance female mouse may "lose" pup and then must return, guided by its squeaks to retreive it carrying it to the nest, where the squeaks stop.



SUMMARY

Distress vocalizations were recorded from deermouse pups of four taxa (<u>Peromyscus maniculatus bairdi</u>, <u>P.m.</u> <u>gracilis</u>, <u>P.leucopus</u>, and <u>P.polionotus</u>) for each day from birth until eye opening. Recordings were also made of four other taxa (<u>P.californicus</u>, <u>P.m.nebracensis</u>, <u>P.melanophrys</u>, and <u>P.difficilis</u>). The vocalizations were analyzed, by means of magnetic tapes, oscillograms, sound spectrograms, and sonagrams, for frequency characteristics, numbers of squeaks in a set, set duration, and squeak duration.

It was hypothesized that 1) the frequency and temporal patterns of the squeaks carry information on the species, age and location of the pup; 2) the characteristics of the squeaks change with age; 3) the squeaks serve as a stimulus for maternal retrieving behavior; 4) they also carry sufficient information to enable retrieving mice to identify species; and 5) the source of these squeaks can accurately be located by the mother mouse.

The results of sound analyses and behavioral tests confirmed these hypotheses, in particular as follows:

1. Temporal parameters; <u>Peromyscus</u> distress squeaks are generally short, sharp sounds uttered in groups. Squeak duration, number of squeaks in a set, and set duration were



characteristically constant within species but differed significantly between species. All of the taxa differed from the others on at least one of these parameters. Numbers of squeaks per set ranged from 3.15 (<u>P.leucopus</u>) to 4.60 (<u>P</u>. <u>melanophrys</u>) squeak duration ranged from 0.13 (<u>P.poliono-</u> <u>tus</u>) to 0.18 sec. (<u>P.m.bairdi</u> and <u>P.californicus</u>); set duration ranged from 0.81 sec. (<u>P.m.nebracensis</u>) to 1.48 sec. (<u>P.californicus</u>).

2. Frequency parameters; Two frequency patterns were found, pure tone squeaks with no visible harmonics (Type I) and squeaks with 2 main bands of sound energy and much intensity modulation (Type II). The first type were found in <u>P.leucopus</u> and <u>P.polionotus</u> and the second mostly in <u>P</u>. <u>difficilis</u>, and <u>P.melanophrys</u> had similar mean basic frequencies (21-24 kHz). while <u>P.polionotus</u> and <u>P.leucopus</u> had higher mean basic frequencies (32-33 kHz) and <u>P.californicus</u> had a low frequency of 18 kHz.

3. Phylogenetic differences; Frequency characteristics were most similar within species. Temporal parameters varied even between subspecies. All species differed on some frequency pattern.

4. Ontogenetic changes; Frequencies tended to rise with age and older pups modulate frequency and intensity less than younger ones. Harmonics gradually disappear with age. Numbers of squeaks per set and set duration decreased and squeak duration increased, peaked and then dropped with age.



5. Recorded squeaks activated lactating <u>P.manicula-</u> <u>tus</u> mothers to leave nests and search for the source of the sound. The mice preferred the squeaks to control sounds.

6. Lactating <u>P.maniculatus</u> and <u>P.leucopus</u> mice slightly preferred recorded squeaks of conspecific pups to those of other species.

7. Recorded squeaks alone provided sufficient information for accurate location of sound sources by lactating <u>P.maniculatus</u> mice. Recorded squeaks were located as accurately as squeaks of live pups.

The results of these tests confirm the popular intuition that the distress cry on an infant mouse carries information to properly primed adults which draws their attention, stimulates them to search for the source of the sound, and enables them to locate it. In addition, the squeaks carry information on species identity, age of the pup and cues for accurate location.

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APPENDICES

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APPENDIX A

Electronic Equipment

The following list and drawing of the recording and playback pathways are included to clarify the electronic setup for comparative purposes.

- 1. Bruël and Kjaer condensor microphone, ½ in., #4133/34; with cathode follower; frequency response rated at 20 Hz - 40 kHz.
- 2. Bruël and Kjaer microphone power supply, #2801.
- 3. Tektronix #129 plug-in power supply with #2A61 plug-in units; used to step up the signal from the microphone to the tape recorder; essentially unlimited frequency response.
- 4. Ampex SP-300 Instrumentation tape recorder (multi-channel, 4 speed), 10½ in. reels; 3 db s/n ratio; frequency response flat 50 Hz 40 kHz. All recording done at 15 ips; playback at 1-7/8 ips for analysis. Ampex Instrumentation tape, 1 ml mylar, #641.
- 5. Tektronix Oscilloscope #3A74.
- 6. Grass Oscilloscope Camera
- Bruël and Kjaer frequency analyzer, #2107, frequency response 20 Hz - 20 kHz; with Bruël & Kjaer logarythmic potentiometer #2305.
- Acoustic spectrograph "Voice Print" #4177B; 85-8000 Hz response, Kay Sonagraph paper type B.





Figure A-1 Legend

Schematic of the electronic recording and analysis pathways. Pieces of equipment are marked and connections are indicated by arrows.





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