ANALYSIS OF ULTRASONIC COMMUNICATION DURING SEXUAL BEHAVIOR IN DEER MICE (*PEROMYSCUS MANICULATUS BAIRDI*)

By

Steven M. Pomerantz

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Psychology and Neuroscience Program

1981

ABSTRACT

ANALYSIS OF ULTRASONIC COMMUNICATION
DURING SEXUAL BEHAVIOR IN DEER MICE
(PEROMYSCUS MANICULATUS BAIRDI)

By

Steven M. Pomerantz

The present study investigated the role of ultrasonic communication by deer mice during sexual behavior. In sexual behavior tests with sexually receptive females, males that copulated always produced 35-kHz ultrasonic vocalizations, whereas males failing to copulate seldom produced ultrasonic vocalizations. There was no evidence to indicate that females produced ultrasounds.

Hormonal status of the female influenced male ultrasound production. Males vocalized with sexually receptive females, but rarely vocalized with ovariectomized females. Moreover, sexually receptive females that were anesthetized elicited male ultrasonic calling.

A courtship role for precopulatory ultrasounds was indicated by the finding that males always produced ultrasounds prior to the onset of copulation. Furthermore, during the period when males were producing precopulatory ultrasounds, increases in male solicitation rate, female locomotor activity, and in the proximity maintained between the male and female were observed. Thus, precopulatory

ultrasounds were associated with both elevated levels of male sexual behavior and female proceptive behavior (i.e., female behavior that facilitates copulation). Male ultrasounds do not appear to play an important role during copulation, since vocalization rate declined during this period, while other measures of male and female sexual behavior remained high. Following ejaculation, male ultrasonic calling resumed despite substantial decreases in other male sexual behaviors. Also, females were very active during this period, indicating that postejaculatory vocalizations may serve to sustain proceptive behavior of the female during a time when the male is in a withdrawn state.

Gonadal hormones influenced male ultrasonic calling. Testosterone, as well as two of its major metabolites, dihydrotestosterone, and estradiol, restored ultrasound production in long-term castrated males. Combined treatment with subthreshold dosages of both dihydrotestosterone and estradiol activated male ultrasonic calling and male copulatory behavior. This observation supports the hypothesis that testosterone may stimulate all aspects of male sexual responding in deer mice by being metabolized to both its reduced metabolite, dihydrotestosterone, and its aromatized metabolite, estradiol.

In conclusion, these experiments suggest that ultrasonic vocalizations by male deer mice are an integral component of their sexual behavior repertoire.

ACKNOWLEDGEMENTS

I offer many thanks to Dr. Lynwood Clemens for his support and good-nature during my graduate career. I also wish to thank Dr. Lauren Harris, Dr. John Johnson, Jr., and Dr. John King for their helpful comments and suggestions regarding my graduate work.

I also gratefully acknowledge the expert technological assis- assistance of Evan Fox, Richard Mark, and Michael Vandenberg. Insightful editorial comments were made by Dr. Gary Dohanich and Dr. Anthony Nunez. My thanks go out to all the members of Dr. Clemens' laboratory. I also recognize the secretarial contribution made by Ms. Elizabeth Keith.

Finally, I wish to affectionately recognize my parents and friends for their encouragement and support of my academic ambitions.

TABLE OF CONTENTS

Page

LIST OF FIGURES iv

LIST OF TABLES v

INTRODUCTION 1

Communication During Rodent Reproductive Behavior 2
Stimuli Capable of Eliciting Ultrasounds 8
Behavior of the Ultrasound Producer and Endogenous Factors Influencing Ultrasound Production 14
Behavioral Responses to Ultrasonic Vocalizations 27
Objectives of the Present Study 34

GENERAL METHODS 36

EXPERIMENT 1 39

EXPERIMENT 2 45

EXPERIMENT 3 49

EXPERIMENT 4 60

EXPERIMENT 5 74

SUMMARY AND CONCLUSIONS 85

REFERENCE NOTES 87

LIST OF REFERENCES 88

LIST OF FIGURES

Figure		Page
1	Mean (±SEM) vocalization frequency and vocalization rate of male deer mice before copulation (PVIL), during copulation (EL), and after ejaculation (PEI)	51
2	Mean (±SEM) solicitation frequency, solicitation rate, and % locomotor activity by vocalizing and copulating male deer mice during different temporal periods of the sexual behavior test	56
3	Percentage of castrated male deer mice exhibiting ultrasonic vocalizations and mounting behavior following treatment with 1 ug EB/day, 2 ug EB/day, 3 ug EB/day, or OIL	63
4	Percentage of castrated male deer mice exhibiting ultrasonic vocalizations, mounting, intromission, and ejaculation following treatment with 50 ug DHTP/day, 100 ug DHTP/day, or 200 ug DHTP/day	65
5	Percentage of castrated male deer mice exhibiting ultrasonic vocalizations, mounting, intromission, and ejaculation following treatment with 1 ug EB/day, 50 ug DHTP/day, 1 ug EB + 50 ug DHTP/day, or 200 ug TP/day . .	68
6	Mean (±SEM) solicitation frequency, solicitation rate, % locomotor activity, and % proximity observed during different temporal periods of the sexual behavior test in sexually receptive female deer mice tested with vocalizing and copulating males	78

LIST OF TABLES

Table		Page
1	Rodent Ultrasonic Calls During Heterosexual Encounters	5
2	Relationship Between Male Copulation and Ultrasonic Vocalizations	41
3	Comparison of Male Solicitation Frequency, Solicitation Rate, and Locomotor Activity in 3 Groups of Male Deer Mice	54
4	Vocalization Performance of Castrated Male Deer Mice Given Various Daily Hormone Treatments	70
5	Influence of Male Behavior on Female Proceptive Behavior in Deer Mice	76

INTRODUCTION

Coordination of male and female sexual behavior is a key requirement for successful reproduction. At appropriate times, both the male and female must be sufficiently motivated and able to perform sex-specific and species-specific sexual behaviors. Obviously, a mating pair does not achieve the synchrony observed in their reproductive behavior by accident or good fortune, instead coordinated patterning of sexual activity is facilitated by communication between the sexual partners.

Individuals of both sexes transmit various signals that advertise their species identity, sexual identity, sexual arousal, sexual and social status, location, and other information important to a prospective mate. The receiver must accurately perceive and integrate the incoming messages so that an appropriate and effective response can be made and the communication process continued. Thus, social signalling occurring in a reproductive context means that both members of a mating pair are assuming and exchanging the roles of sender and receiver of messages. In this fashion, natural selection would be expected to operate on both sexes of the communicating dyad to yield "co-evolved, bidirectional signal production and signal reception" (Green & Marler, 1979).

Communication is not restricted to one modality. Depending on the species and the ecological constraints on that species, various social signals may be appropriate. As noted by Kelly (1980), "Species diversity in signalling modalities may be viewed as adaptations for effective communication in different habitats" (p.111). Certain signals are better suited for communication in certain situations than others. For example, acoustic and chemical signals are generally able to be transmitted and received over longer distances than visual or tactual signals (Marler, 1976).

Communication During Rodent Reproductive Behavior

Various modes of communication are important for rodent reproductive behavior (Note 1). Although tactile and olfactory signalling will be briefly covered in this discussion, the major emphasis of this review will be on the role of ultrasonic communication (>20 kHz) in rodent sexual behavior.

Somatosensory stimulation provided by both partners is extremely important for reproduction. Tactile stimulation of the female's flanks, rump, and tailbase by the male has been found to be both necessary and sufficient for eliciting the lordosis reflex (Pfaff, 1980). Lordosis, in turn, promotes genital stimulation of both sexes; thereby, faciltating vaginal entry of the male's penis, sperm transport following ejaculation, and subsequent fertilization of the ova (Adler, 1978).

Tactile stimulation is obviously essential for directing copulatory mounts by the male. However, prior to the initiation of

copulation, messages must be sent which serve to bring the sexes together. Whether by glandular secretion, marking, or urination, males and females of all rodent species studied send chemical signals. There is good evidence to suggest that these chemosignals function to attract and arouse conspecific mates (Bronson & Caroom, 1971; Caroom & Bronson, 1971; Doty, 1972; Johnston, 1974; 1975; 1979; Murphy, 1973). Moreover, the possibility that the hormonal status of the animal is being communicated by these chemosignals seems likely, since both the chemical composition of the odors and the behaviors necessary for odor emission have been found to be regulated by gonadal hormones (Bronson, 1971; Johnston, 1977; 1979; Thiessen, Friend & Lindzey, 1968). The importance of olfactory stimulation was further demonstrated by studies in which olfactory impairment was produced experimentally. Complete bilateral olfactory bulbectomy of males in many different rodent species resulted in severe disruption or elimination of mating (reviewed by Murphy, 1976). In females, preventing olfactory stimulation apparently does not interfere with performance of lordosis, but deficits in precopulatory or courtship behavior have been noted (reviewed by Murphy, 1976).

The ability of muroid rodents to produce and hear sounds above 20 kHz is well-established (Brown & Pye, 1976; Nyby & Whitney, 1978). Ultrasonic vocalizations occur in a variety of social settings. For example, studies demonstrated that ultrasonic communication is important during mother-infant interactions (Allin & Banks, 1971; 1972; Bell, 1974; Bell, Nitschke, Gorry, & Zachman, 1971; Bell, Nitschke, Zachman, 1972; Colvin, 1973; Smotherman, Bell, Starzec, Elias, & Zachman, 1974). Separation from the home nest, cold stress,

rough handling by the dam, and presence of novel conspecific odors are all stimuli which elicit ultrasound production from infant rodents. These ultrasonic calls, in turn, signal the mother to modify her maternal behavior (reviewed by Bell, 1974; 1979; Noirot, 1972; Sales & Smith, 1978).

Among adult rodents evidence suggests that ultrasonic signalling infuences animal aggressive behavior, territoriality, alarm behavior, and reproductive behavior (reviewed in Nyby & Whitney, 1978). The present discussion will concentrate on the importance of ultrasonic communication occurring within the context of reproductive behavior. Occassionally, it will be necessary to discuss and compare the role of adult ultrasounds in this social context with that found in other social contexts.

Table 1 summarizes the heterosexual situations in which ultrasounds have been detected in 13 rodent species, the sex or sexes producing vocalizations, and the physical characteristics of the vocalizations. Depending on the species, ultrasounds have been detected during one or all of the following time periods of a mating sequence: (1) precopulatory or courtship - time from introduction of mating pair until first mount; (2) copulatory - time from first mount until ejaculation; and (3) postejaculatory - time from ejaculation until the next mount of the following copulatory species (Nyby & Whitney, 1978). Species differences with respect to the sex of the animal producing ultrasounds have also been noted. In species such as golden hamsters and collared lemmings, both sexes participate in ultrasound production (Brooks & Banks, 1973; Floody, Pfaff, & Lewis, 1977), while in species such as house mice, rats, and Mongolian

TABLE 1

RODENT ULTRASONIC CALLS DURING HETEROSEXUAL ENCOUNTERS

SPECIES	PHYSICAL CHARACTERISTICS duration (ms)	central frequency (kHz)	SEX PRODUCING CALL	SITUATION DURING WHICH CALLS PRODUCED	AUTHORS
FAMILY MURIDAE					
Mus musculus	50-300	70	male	precopulatory copulatory	Sales (1972) Whitney Stockton & Tilson (1971)
Rattus norvegicus	100-500	40-60	male	precopulatory copulatory	Sales (1972) Geyer & Barfield (1978) McIntosh & Barfield (1980)
	1000-3000	22	male	preejaculatory ejaculatory	Brown (1979) Barfield & Geyer (1972) Anisko, Suer McClintock & Adler (1978)
Acomys cahirinus	100-300	50-60	?	precopulatory copulatory	Sales (1972)
Apodemus sylvaticus	2-40	75-85	?	precopulatory copulatory	Sales (1972)

TABLE 1 (cont)

SPECIES	PHYSICAL CHARACTERISTICS duration (ms)	central frequency (kHz)	SEX PRODUCING CALL	SITUATION DURING WHICH CALLS PRODUCED	AUTHORS
Mus minitoides	2-30	80-90	?	precopulatory	Sales (1972)
Praomys natalensis	5-30	70-85	?	precopulatory	Sales (1972)
FAMILY CRICETIDAE					
Mesocricetus auratus	80-100	34-42	female	precopulatory	Floody & Pfaff (1977)
	70-150	32-38	male	precopulatory	
Lagurus lagurus	15-60	80	?	precopulatory copulatory	Sales (1972)
Callomys callosus	2-25	65-70	?	precopulatory copulatory	Sales (1972)
Peromyscus maniculatus	2-25	35-60	?	precopulatory copulatory	Sales (1972)
Clethrionomys glareolus	2-25	20-25	?	copulatory	Sales (1972)
Dicrostonyx groelandicus	70	15-35	female	precopulatory	Brooks & Banks (1973)
	70	15-35	male	precopulatory copulatory	

TABLE 1 (cont)

SPECIES	PHYSICAL CHARACTERISTICS duration (ms)	central frequency (kHz)	SEX PRODUCING CALL	SITUATION DURING WHICH CALLS PRODUCED	AUTHORS
Meriones unguicalatus	19-56 145	28-38 26	male	precopulatory copulatory postejaculatory	Holman (1980)

gerbils, the male is primarily responsible for ultrasonic vocalization (Holman, 1980; McIntosh, & Barfield, 1980; Sales, 1972; Whitney, Coble, Stockton, & Tilson, 1973).

Stimuli Capable of Eliciting Ultrasounds

Ultrasonic vocalizations generally do not occur spontaneously, but appear as an animal's response to a particular social situation. In sexual encounters, signals being sent by one sex have been found to directly elicit or influence ultrasonic emmission of the opposite sex. Comparative research has revealed interesting similarities and differences between species concerning the nature and manner in which environmental stimuli elicit ultrasound production.

House Mice

Adult males produced a greater number of ultrasounds when placed with an adult female than when placed with another adult male (Whitney et al., 1973). Investigations into the signals from adult females which are necessary or sufficient for eliciting male ultrasounds revealed that males vocalized in response to the presentation of soiled bedding from an adult female (Whitney, Alpern, Dizinno, & Horowitz, 1974). Thus, chemical cues contained in the soiled bedding were sufficient to elicit male ultrasounds whereas, visual, auditory, or movement signals apparently were not necessary. Subsequently, it was discovered that female mouse urine contained the necessary ultrasound-eliciting properties, but male mouse urine did not (Nyby, Wysocki, Whitney, & Dizinno, 1977b).

Experiments designed to test factors which might influence the signal value of female urine revealed that female urine probably acquires its ultrasound-eliciting properties when the female reached puberty (Nyby, Wysocki, Whitney, Dizinno, & Schneider, 1979). Neonatal or adult castration of male mice did not improve the abilty of their adult urine to stimulate significant ultrasound production. Also, neither androgen administration to neonatal females nor adult castration of females adversely affected the abilty of their adult urine to elicit ultrasounds. The importance of the pituitary in mediating the ultrasound-eliciting cues in female urine was demonstrated by the observation that female urine from hypohysectomized females failed to stimulate male ultrasound production. Recent evidence suggests that neither FSH nor LH alone, but rather a combination of FSH and LH is necessary for female urine to acquire its signal value (Nyby, personal communication).

The signal value of female urine may be learned by males. Adult males that were separated from females at weaning did not initially emit ultrasounds in response to female urine (Dizinno, Whitney, & Nyby, 1978). Males that were similarly isolated from females at weaning, but as adults provided with brief exposure to a female (e.g. one 3-minute exposure), subsequently emitted ultrasounds in the presence of female urine (Dizinno et al., 1978; Nyby et al., 1977b). Moreover, in these socially experienced males that had acquired the ability to produce ultrasounds, repeated testing with female urine resulted in extinction of the ultrasound response (Dizinno et al., 1978). Thus, experiential factors influence the ultrasonic responsiveness of males. In fact, using classical conditioning

paradigms, artificial odors could acquire signal value (Nyby, Whitney, Schmitz, & Dizinno, 1978). This was demonstrated by placing perfume on the mother prior to weaning or on adult females used to provide the male with his first heterosexual experience. When the males were tested for ultrasound production to the perfume, only males that had previously encountered perfume as adults on adult female mice emitted ultrasounds. These data support the notion that female urine acquires its signal value as a result of adult experience rather than through neonatal imprinting.

Paradoxically, some results of male ultrasounds in tests using stimulus animals appear to contradict the results obtained using urinary stimuli alone (Nyby, Wysocki, Whitney, Dizinno, Schneider, & Nunez, 1981). Adult castrated males, neonatally castrated adult males, hypophysectomized males, prepubertal females, and hypophysectomized females all elicited ultrasonic vocalizations, whereas urine from these same stimulus animals was ineffective in stimulating ultrasounds (Nyby et al., 1979). The authors suggest that the strategy of the male is to produce courtship vocalizations to another mouse regardless of sexual, hormonal, or chemosensory status. Depending on the ensuing behavioral interactions, ultrasounds will either be inhibited (e.g., during aggression) or maintained (e.g., during sexual behavior). One criticism of this research on the signal value of stimulus animals is that the tests were only 3 minutes in duration. Therefore, failure to find the same ultrasound-eliciting properties in stimulus animals as were found using urinary stimuli could be related to a failure to give the males sufficient time to

recognize behavioral differences which might have existed among the different stimulus animals.

Laboratory Rats

Male rats produce two types of ultrasounds. Fifty kHz vocalizations occur during courtship and copulatory behavior, whereas 22-kHz vocalizations occur during a variety of social situations including aggression, following ejaculation, and occassionally following a period of courtship or preejaculatory 50-kHz calling (Barfield, Auerbach, Geyer, & McIntosh, 1979). In contrast to male mice, stimulation of both 50- and 22-kHz male rat calls is greatly influenced by the hormonal state of the female (Geyer & Barfield, 1978). Males tested with ovariectomized females exhibited low rates of calling. Estrogen treatment of ovariectomized stimulus females led to a higher rate of production of both 50- and 22-kHz calls, but maximal production was observed when males were tested with females receiving both estrogen and progesterone.

Olfactory cues being produced by receptive females are apparently important in eliciting male ultrasounds. Males vocalized in the presence of an anesthetized receptive female or the soiled cage shavings from an estrus female (Geyer & Barfield, 1978). In contrast, anesthetized ovariectomized (OVX) females were not effective in eliciting ultrasounds. An interesting finding in this study was that 50-kHz ultrasonic vocalizations were emitted for a longer period by males tested with an awake OVX female than by males tested with an anesthetized estrous female. These results indicate that in addition to olfactory signals, other signals (e.g. visual, auditory, tactile)

which females emit during behavioral interactions with males play a significant role in maintaining male ultrasound production. The influence of signals other than olfactory cues appears particularly important for male preejaculatory 22-kHz vocalizations. These 22-kHz vocalizations were produced by males in response to females that were exhibiting decreased lordosis intensity and increased aggressiveness toward the male (Brown, 1979).

Golden Hamsters

In golden hamsters, both males and females produce ultrasonic vocalizations. Following presentation of a stimulus male, estrous females emitted ultrasounds (Sales, 1972). Moreover, olfactory cues provided by either male cage shavings or an anesthetized male stimulated female ultrasound production (Floody, Pfaff, & Lewis, 1977). Also, auditory stimuli elicited female ultrasonic signalling (Floody & Pfaff, 1977b), as demonstrated by the observation that estrous females vocalized in response to synthetic tape recordings of male calls. Experience was found to influence female calling. Estrous females given repeated social experience with males began to anticipate the presence of the male by increasing their rate of calling immediately prior to the introduction of the male (Floody et al., 1977).

Male hamsters that produced ultrasonic vocalizations to an estrous female were observed to continue vocalizing after the removal of the female from the test cage (Floody & Pfaff, 1977a). However, physical characteristics of the call differed in the two situations. In the female's presence, male vocalizations tended to have lower

minimum frequencies, higher maximum frequencies, longer durations, and fewer rapid frequency changes than when the male was alone following the female's removal. Similar to rats, male hamster ultrasounds are influenced by the hormonal state of the female (Floody et al., 1977). Males that were paired with estrous females vocalized significantly more often than males paired with diestrous females. Also, similar to both rats and house mice, olfactory cues provided by anesthetized females elicited male vocalizations (Floody et al., 1977). However, males did not differentially vocalize to anesthetized females which were in different hormonal states, as they had done with awake females in different hormonal states. These results indicate that males may discriminate between estrous and nonestrous females on the basis of behavioral cues (e.g., female ultrasonic vocalizations, lordosis, or aggression).

Other Species

In other rodent species that produce ultrasounds during heterosexual encounters (see Table 1), research on cues that elicit ultrasounds is not extensive. In general, males appear to vocalize in response to females. Also, as has been found in other species, associative learning may be important in determining the stimuli that elicit male vocalizations. For example, after repeated testing, male collared lemmmings would begin producing ultrasounds with the approach of the experimenter and, occassionally, would attempt to mount the experimenter's gloved hand (Brooks & Banks, 1973). Estrous female collared lemmings, in contrast, did not appear to need any cues to elicit ultrasonic calling, but rather they spontaneously vocalized in

their cage (Brooks & Banks, 1973). In other words, these females seem to advertise their behavioral receptivity ultrasonically.

Behavior of the Ultrasound Producer and Endogenous Factors Influencing Ultrasound Production

By observing the behavior of vocalizing animals which occurs in association with ultrasound emission, additional information can be obtained concerning the cues that trigger ultrasound production. For example, if an animal vocalizes following investigation of another stimulus animal, but not while alone, it is reasonable to assume that cues from the stimulus animal triggered ultrasound production. However, it is important to recognize that internal as well as external referents trigger ultrasonic vocalizations. Consequently, all the necessary external cues that elicit ultrasounds may be present, but still an animal may refrain from vocalizing. Alternatively, there are situations in which no apparent external referents are present but, nonetheless, an animal vocalizes to advertise some aspect of its internal state. Green and Marler (1979) hypothesized that an animal utilizes both internal and external referents to form a "signal-generating assessment". On the basis of this assessment a signal and the behavior accompanying it may or may not be produced. In this section, endogenous factors which influence ultrasound production are discussed, as well as the behaviors that are associated with ultrasounds. Each species will be discussed seperately; however, since relatively little research has been done on

neural mechanisms involved in ultrasound production, information on neural mechanisms from the different species will be pooled.

House Mice

In house mice, male ultrasounds were more prevalent during courtship periods than during any other stage of a sexual encounter (Nunez & Bean, unpublished observations; Sales, 1972). Precopulatory ultrasonic pulses occurred during male approaches toward the female and male anogenital investigations of the female. In contrast, ultrasounds were rarely detected when males were involved in maintenance activities such as eating, drinking, or self-grooming. During the initial stages of copulation, ultrasounds often occurred simultaneously when the male mounted the female. As copulation progressed, male ultrasonic vocalizations declined during intromissions and ceased by the time the male ejaculated (It was not determined whether, following an ejaculation, males vocalize before initiating subsequent mating sequences).

Interestingly, the production of courtship ultrasounds seems to be influenced by the dominance status of the male (Nyby, Dizinno, & Whitney, 1976). Socially dominant males responded quicker to the presence of a female with ultrasounds and called significantly more often than subordinate males. This finding is especially important in light of reports which indicate that in house mice, dominant males sired vastly more offspring than subordinate males (DeFries & McClearn, 1970). Quite possibly, dominant males communicate both their social status and their sexual motivation by emitting ultrasounds. Consequently, females can select males on the basis of

ultrasound production. Female responses to male ultrasounds will be discussed later.

As might be expected from a behavior that is closely associated with sexual behavior, male house mice ultrasounds are strongly influenced by gonadal hormones. Both the latency to the first ultrasound and the number of ultrasounds produced by adult males declined following castration (Dizinno & Whitney, 1976; Nunez, Nyby, & Whitney, 1978). Subsequent testosterone (T) administration to castrated males restored ultrasonic vocalizations. Furthermore, a recent study by Nunez et al., (1978) indicates that T may exert its stimulatory effects in part by aromatization (conversion) to estradiol (E). E restored ultrasonic vocalizations in a significant proportion of castrated males. However, E treatment did not consistently support ultrasound rates comparable to those achieved by T-treated males. In contrast, dihydrotestosterone (DHT), a reduced androgenic metabolite of T, did not stimulate ultrasound production when administered alone and, when administered in combination with E, did not significantly enhance the call rates over animals receiving E alone.

In house mice the effect of sex hormones on ultrasonic vocalizations appears to be predominantly "activational" rather than "organizational". This conclusion is based on the finding that females, which were ovariectomized as adults and treated with T, emitted many more ultrasounds to stimulus females than either sham or OVX females treated with OIL (Nyby, Dizinno, & Whitney, 1977a). In fact, T-treated OVX females exhibited a level of ultrasound production comparable to T-treated castrated males. Evidently, adult females possess the potential to exhibit ultrasounds, but normally do not

vocalize because they lack the necessary hormonal substrate as adults.

Laboratory Rats

Similar to house mice, male laboratory rats produce precopulatory ultrasounds (50-kHz) during solicitations and anogenital investigations of the female (Sales, 1972). McIntosh and Barfield (1980) examined the association between male vocalizations and male copulatory behavior. Although the study is flawed in several respects related to the data analysis, it represents the first attempt to investigate the precise temporal relationship of male ultrasonic vocalizations to events which occur during mating. Males vocalized in bursts or clusters, termed "vocalization bouts", immediately preceding (i.e. 10 sec before) most mounts, intromissions, and ejaculations. The highest number of vocalizations occured before an ejaculation. In addition, more vocalizations preceded an intromission than preceded a mount. In contrast to the vocalization bouts, following a mount or intromission there was a discrete period of time, occupying 90-95% of the interval between intromissions, during which no 50-kHz vocalizations were produced. This "vocalization pause" was characterized by autogenital grooming by the male and, further supports the notion that males experience a period following an intromission during which very few sexual behaviors are exhibited (Sachs & Barfield, 1970).

During copulation (i.e. ejaculation latency-time from first intromission to ejaculation), males that mated quickly (short ejaculation latency and short intervals between intromissions) vocalized less frequently than slow maters. This relationship seems

paradoxical. If it is assumed that vocalization rate is positively related to male copulatory rate, then one would predict fast maters to exhibit high vocalization rates. McIntosh and Barfield (1980) offer two explanations to account for this curious relationship between rates of vocalization and rates of copulation:

> (1) It is possible that high levels of precopulatory vocalizations are associated with both sexual arousal and the facilitation of male-female investigatory responses, but that once copulation has begun, fewer vocalizations are necessary to main- sexual excitation between the 'aroused' maters and the female ... (2) The fact that the males who took longer to ejaculate produced more vocalizations than the faster maters suggests that the females may have been behaving differently in in these tests. Indeed female solicitation rates (amount of darting/unit time) were found to be much lower in those tests involving slower maters. The possibility exists then, that the behavior of the female is directly influencing ultrasonic vocalization production of the male. The increase in 40- to 60-kHz vocalizations in those tests involving slower maters might therefore reflect increased arousal levels in sexually excited animals in response to an uncooperative mating partner. (p.354-355).

Regarding the first hypothesis, there are, unfortunately, no data presented on whether precopulatory vocalization rate varied from the vocalization rate observed during copulation (i.e. ejaculation latency). However, the alternative hypothesis is intriguing in that it demonstrates the complexity involved in behavioral analysis of communication. Evidently, male ultrasound performance is not a "fixed action pattern" which is simply elicited by certain cues. Rather males appear to adjust and modulate their ultrasound and sexual behavior performance as a result of the quality of the female response to previous ultrasounds. The topic of responses to ultrasonic vocalizations will be discussed in detail later.

Much research has been done on the 22-kHz vocalizations of male rats. Males produce this call in a variety of social situations

including submission in aggressive encounters (Lore, Flannelly, & Farina, 1976; Sales, 1972b; 1979)., before and after ejaculation (Anisko, Suer, McClintock, Adler, 1978; Brown, 1979; Barfield & Geyer, 1972; 1975; Pollack & Sachs, 1975) and, occassionally, following bouts of 50-kHz precopulatory calling (Geyer & Barfield, 1978; Geyer, Barfield, & McIntosh, 1978a). Recently, Adler and Anisko (1979) reviewed these studies and suggested that although the specific meaning or social function of the 22-kHz call varies in different social contexts, the message or information provided by the call may be similar. By emitting this call, the male rat conveys information about his motivational state; specifically, that he is in a withdrawn, refractory, or helpless condition.

Adler and Anisko (1979) tested the idea that in a variety of behavior contexts a male rat in a "helpess state" will produce 22-kHz calls. They found that 22-kHz calling followed both ejaculation and inescapable shock treatment. Moreover, after ejaculation, males behaved in a fashion similar to males that were in a state of learned helplessness (Maier & Seligman, 1976).

Physiological evidence also supports the notion that during the postejaculatory period of 22-kHz calling, male rats have entered a refractory state. They exhibited a high-amplitude, slow wave EEG activity characteristic of sleep (Kurtz & Adler, 1973; Barfield & Geyer, 1975), reductions in locomotion and general activity (Dewsbury, 1967), and increases in urination (Anisko, Adler, & Suer, 1979). These are all signs of parasympathetic activity which indicate that the animal is in a "vegetative" state (Adler, 1974).

Although the state of males producing 22-kHz calls after defeat in an agonistic encounter may be similar to that following ejaculation, it does not seem apparent that similar motivational states underly the pre- and postejaculatory calling of males. Before ejaculation, males are active rather than being socially withdrawn. Nevertheless, males emitted 22-kHz calls prior to ejaculation, after they had already had several ejaculations with a female (Anisko et al., 1978; Brown, 1979). These preejaculatory vocalizations were associated with an increase in mounts without intromissions and appeared to occur in response to decreased lordosis intensity and increased aggressiveness of the female. Thus, it has been suggested that even in this situation, the 22-kHz vocalizations resulted from both the frustration and conflict (i.e. helplessness) that males experienced in mating with uncooperative females (Adler & Anisko, 1979).

Very little research has been done on hormonal factors mediating male rat ultrasonic calling. Similar to house mice, castration results in a decline in both 50-kHz and 22-kHz vocalizations (Geyer et al., 1978a; Parrott, 1976). There is no information on gonadal hormone replacement and restoration of 50-kHz vocalizations. Interestingly, T activated postejaculatory 22-kHz vocalizations in both castrated males and females exhibiting ejaculatory patterns (Barfield & Krieger, 1977; Parrott & Barfield, 1975). However, the calls of the T-treated females tended to be shorter and less reliably produced than the calls of T-treated males. The full capacity to exhibit male-like postejaculatory 22-kHz calls appears to depend on perinatal exposure to androgens, since females given T neonatally

exhibited ejaculatory reflexes and postejaculatory vocalizations which were indistinguishable from normal males (Barfield & Geyer, 1975).

Golden Hamsters

Although both male and female hamsters emit ultrasounds (35-kHz), the physical characteristics of these calls differs between the sexes (Floody & Pfaff, 1977a). Male calls tended to have fewer abrupt changes in amplitude and frequency than female calls. Also, the mean duration of male ultrasonic calls was nearly twice that of female calls.

During initial hetrosexual contact before the onset of copulation, both sexes exhibited high rates of ultrasonic production (Floody et al., 1977). Female ceased ultrasound production once they assumed lordosis and the rate of ultrasound production by males declined dramatically. Unfortunately, hamster vocalizations have yet to be monitored throughout a copulatory session. It would be interesting to determine whether male hamsters changed their rate of production as sexual behavior progressed in a manner similar to male rats and house mice. Regarding this suggestion, it is important to realize that although sexual dimorphism in physical characteristics of hamster ultrasonic vocalizations was noted in individual sound spectograms, male and female ultrasounds monitored over a long period of time appeared identical. Thus, any consideration of the relationship between vocalization rate and sexual behavior would be confounded by the contribution of both sexes to the vocalization paramater. However, this problem might be able to be solved by using methods of devocalization that have recently become available.

Gonadal hormones strongly influence male hamster ultrasonic vocalizations (Floody, 1981; Floody, Walsh, & Flanagan, 1979b). Castration resulted in a decline in ultrasound production; this decline was reversed by T-propionate (TP) treatment (Floody et al., 1979b). Castrated males given TP vocalized in several situations: (1) when placed with a receptive female in lordosis; (2) following the female's removal; and (3) in response to synthetic ultrasounds.

Recent preliminary data indicate that E, an aromatized metabolite of T, was effective in maintaining ultrasound production in castrated males, but was not nearly as effective as T (Floody & Merkle, unpublished). In contrast, DHT, a reduced metabolite of T, did not maintain ultrasound production. At present these data are not conclusive since only one dose of each hormone was used. Nevertheless, the results are consistent with other studies which suggest that aromatization of androgens plays a greater role in the hormonal control of male mating behavior in hamsters than reduction of androgens (DeBold & Clemens, 1978; Whalen & DeBold, 1974). It is important to realize; however, that reduced androgens may be involved in male hamster ultrasonic vocalizations since it was recently demonstrated that DHT and E can act synergistically to control male hamster copulatory behavior (DeBold & Clemens, 1978).

Similar to males, female hamster ultrasound production is regulated by their endocrine state. Female hamsters produced more ultrasounds to male stimuli during estrus than during other periods of the estrous cycle (Floody et al., 1977). The importance of ovarian steroids in controlling female ultrasonic vocalizations was more convinicingly demonstrated in a study in which OVX females were tested

with various steroid hormone treatments (Floody, Merkle, Cahill, & Shopp, 1979a). Daily treatment with E alone maintained moderates rates of ultrasonic emission that were greater than the rates observed in progesterone (P) or oil-treated females. Maximal call rates were activated by combined treatment with E for 3 days followed by 1 day of P. In the same study, E or P alone failed to stimulate the female lordosis response, whereas the combination of E + P resulted in maximal levels of receptivity. Although ultrasound production and lordosis in female hamsters seem similarly mediated by ovarian hormones, there appear to be subtle differences in the control of the two behaviors. E alone is necessary and sufficient for ultrasound production, whereas E stimulated lordosis only when it was combined with P.

Other Species

In other species, ultrasonic communication during sexual encounters is genarally correlated with courtship and copulatory behaviors (Sales & Pye, 1974). Two species, collared lemmings and gerbils, have been studied fairly extensively.

In a monograph by Brooks and Banks (1973), ultrasounds were detected in 70% of tests between male and estrous female collared lemmings. Sexual behavior occurred regardless of whether there was ultrasonic communication. However, animals in tests with ultrasounds tended to exhibit shorter latencies to initiate both male (mounting) and female (lordosis) sexual behaviors. Although both sexes generally vocalize during sexual encounters, females were observed to emit the first ultrasound in 80% of these encounters. Possibly female collared

lemmings advertise their sexual receptivity ultrasonically. Such a notion is supported by the finding that 21 out of 24 estrous females produced ultrasounds spontaneously while in their home cage, whereas spontaneous calls were never detected from diestrous females. In addition to calling, estrous females typically initiated sexual behavior by soliciting, attacking, and mounting the male. Following the occurrence of these "male-like" behaviors by the female, males usually became sexually aroused and started to produce ultrasounds while chasing, grooming, and mounting the female. Once the pair began copulating, males continued to vocalize at high rates and females ceased ultrasound production. Immediately upon ejaculation, all ultrasonic communication stopped. Interestingly, after an unspecified period of time following ejaculation, females were reported to reinitiate sexual activity by emitting ultrasounds while solicting the male.

Ultrasound production in Mongolian gerbils is sexually dimorphic with males being primarily responsible for vocalization (Holman, 1980). Males produce three physically different types of ultrasounds during sexual interactions. Low intensity, frequency upswing vocalizations were produced during precopulatory and copulatory periods. Frequency modulated vocalizations were produced only during copulation. Finally, a high-intensity unmodulated tone was emitted after ejaculation.

Recently, the influence of neonatal gonadal hormones on the organization of these sexually dimorphic gerbil vocalizations was studied (Holman, 1981). Males castrated on the day of birth showed severe deficits in ultrasound responding despite the fact that they

were given TP as adults. Furthermore, adult females, which were administered TP, both on the day of birth and as adults, exhibited all 3 types of "male" ultrasounds when paired with receptive females. These results clearly demonstrate that gonadal hormones can influence the organization of ultrasound behavior in Mongolian gerbils.

Neural Mechanisms Involved in Ultrasound Production

Since in all rodent species studied, ultrasonic vocalizations are elicited by exposurse to conspecific odors of the opposite sex, several investigators have attempted to determine whether ultrasound production is affected by disruption of olfactory systems. In both male house mice (Bean, in press) and female hamsters (Kairys, Magalhaes, & Floody, 1980) ultrasound responding to stimulus animals was suppressed by olfactory bulbectomy. Additionally, house mice exhibited a reduction in ultrasonic vocalizations after receiving vomeronasal tract cuts, but not intranasal ZnSO flush which eliminates only main olfactory bulb imput (Bean, in press). These results not only confirm the prediction that olfactory stimulation is essential for eliciting ultrasound production, but also suggest that the vomeronasal system mediates the ultrasonic response to chemosignals by serving as the primary system involved in the perception of these signals.

There is clearly a strong association between ultrasonic vocalizations and sexual behavior. Consequently, neural systems that control ultrasound production may also be involved in the control of other reproductive behaviors. In contradiction to this suggestion, medial preoptic lesions in male mice were found to severely disrupt

male copulatory behavior without altering either male anogenital exploration of the female or male ultrasonic vocalizations (Bean, Nunez, & Conner, 1981). These data indicate that coupling of courtship and copulatory behaviors most likely does not occur in the medial preoptic area.

Nevertheless, there is also evidence that neural sites of overlap exist, so that integration of courtship and copulatory behaviors may take place. One such site is the mesencephalic central grey. Gonadal hormones are heavily concentrated by cells in this region (Pfaff & Keiner, 1973; Sar & Stumpf, 1977). Furthermore, female hamsters with lesions in this area exhibited a decline in the incidence of both ultrasonic vocalizations and lordosis responding (Floody & O'Donohue, 1980). Additionally, male rats were observed to vocalize ultrasonically following electrical stimulation of the central grey (Yajima, Hayashi, & Yoshi, 1980). Research on the effects of either stimulation or lesion of this region on male copulatory behavior have yet to be performed, but in female rats, electrical stimulation of the central grey facilitated lordosis responding (Sakuma & Pfaff, 1979a), whereas lesions disrupted lordosis (Sakuma & Pfaff, 1979b).

Since research on the neural control of ultrasonic vocalizations is so recent, there are obviously large gaps in our knowledge of this area. A productive research strategy which has recently begun is to determine the motor pathways necessary for ultrasound output and then proceed to anatomically trace the brain sites and pathways which are necessary for ultrasound production. Studies on house mice (Pomerantz, Nunez, & Bean, in preparation), rats (Roberts, 1975; Wetzel, Kelly, & Campbell, 1980), and gerbils (Thiessen, Kittrell, &

Graham, 1980) have all confirmed that the inferior laryngeal nerves innervating the larynx are primarily responsible for the motor output resulting in ultrasonic vocalizations. Bilateral transection of these nerves resulted in a complete elimination of ultrasounds. Even unilateral transection produced dramatic deficits in ultrasound responding. The inferior laryngeal nerves originate in a discrete region of the nucleus ambiguus (Wetzel et al., 1980) that has been found to receive direct projections from the mesencephalic central grey (Jurgens & Pratt, 1979). At present, it is uncertain whether the central grey is the only region of the brain sending fibers directly to the nucleus ambiguus. Nonetheless, the central grey does receive afferents from other brain sites which have been implicated in the control of sexual behavior (Conrad & Pfaff, 1976a; 1976b; Jurgens & Pratt, 1979; Pfaff, 1980). These areas include the corticomedial and basolateral amygdala, septal area, medial preoptic area, anterior hypothalamus, and ventomedial hypothalamus. It seems that with this anatomical evidence the door has now been opened for future physiological studies on the neural mechanisms involved in ultrasound production.

Behavioral Responses to Ultrasonic Vocalizations

Hypotheses regarding the functions of a particular communicatory signal are developed by studying the consequences which follow performance of that signal. These consequences include changes in the behavior of both the producer and receiver of the signal. Behavioral consequences of ultrasound production for the vocalizing animal were

previously discussed. This section considers the effects of ultrasound production on the responses made by recipient animals. Bell (1974) has suggested that the primary effect of ultrasounds on recipient animals is to change their level of arousal. However, many other investigators have argued that ultrasonic vocalizations may trigger a much more specific behavioral response than a genaral alteration in arousal.

House Mice

Several researchers have proposed that ultrasonic vocalizations serve a courtship function in this species (Sales, 1972; Whitney et al., 1973). This proposal is based on several indirect lines of evidence. First, as was discussed previously, male ultrasounds occurred primarily before the onset of copulation and waned as copulation progressed (Sales, 1972). Additionally, male ultrasounds may facilitate copulation by inhibiting female aggression (Sales, 1972). This suggestion is especially relevant if most matings in nature occur during postpartum estrus (Whitney et al., 1973), a time when females are very protective of their pups and extremely aggressive toward strange males (Noirot, Goyens, & Buhot, 1975). Conceivably, by mimicking infant pup ultrasounds, adult males reduce the tendency of females to attack them and, consequently, increase the probability that females will approach and investigate them. Unfortunately, no studies have been published on the responses of either estrous females or postpartum estrous females to male ultrasounds. However, preliminary observations by Nunez and Bean (personal communication) indicated that postpartum estrous females did

not reduce their aggression in the presence of vocalizing males. Furthermore, infant pups were not observed producing ultrasonic vocalizations until after the postpartum estrous period of the dam (Noirot, 1966). These observations undermine the notion that adult male mice utilize ultrasonic vocalizations chimerically so that they can mate with postpartum estrous females.

Conclusive statements regarding the function of adult male mice ultrasounds await further research. Nevertheless, as noted by Marler (1976), the responses to signals are often quite subtle. In fact, "some signals function not so much to impose a qualitative change on the behavior of the respondents, but rather to select a particular class of respondents that may already be predisposed to perform the response in question." (p.61). Such may be the case for house mice ultrasonic communication. Recently, it was discovered that females in natural and artificially induced estrus, but not OVX females, exhibited a preference for vocalizing males over non-vocalizing males (Nunez, Bean & Pomerantz, in preparation). If one also considers that only socially dominant male mice produce ultrasonic vocalizations (Nyby et al., 1976), then the adaptive significance of ultrasonic communication in house mice becomes apparent. Dominant males may use ultrasonic vocalizations to communicate their sexual intentions to the females that are present. Estrous females, in turn, can identify, approach, and remain near males that are both sexually interested in them and reproductively fit (DeFries & McClearn, 1970).

Laboratory Rats

The results of several studies support the idea that 50-kHz vocalizations of male rats promote behaviors of the female that facilitate mating. Estrous females, which were exposed to 50-kHz vocalizations immediately before testing, exhibited a shorter latency to dart (solicit) and a higher rate of darting than estrous females that were not provided this pretest experience (Geyer, McInntosh, & Barfield, 1978b). Additionally, vocalization priming of the female resulted in shorter male intromission latencies.

Other studies have attempted to elucidate more directly the function of 50-kHz vocalizations by studying the effects of deafening females or muting males (Barfield, Auerbach, Geyer, & McIntosh, 1979; McIntosh, Barfield, & Geyer, 1978; Thomas, Howard, & Barfield, 1981). After being deafened, estrous females exhibited a reduction in darting responses to males that were tethered (Barfield et al., 1979). However, deafening did not interfere with the latency to return and accept intromissions from a tethered male. A recent study investigated the mating choice of females during tests with two tethered males, one of which was devocalized (Thomas et al., 1981). More solicitations were directed toward the intact vocalizing male than toward the muted test partner. However, ultrasonic vocalizations did not influence the number of visits or the duration of visits to either the vocalizing or muted male. These findings indicate that the principle function of 50-kHz male rat vocalizations is to facilitate female solicitations. Interestingly, these vocalizations apparently do not serve to maintain females in close proximity to vocalizing males.

According to the semiotic theory of communication (Smith, 1979), although the message of 22-kHz calls by male rats appears to be similar in different social situations (i.e. declaration of social withdrawal), the specific meaning or function of the call depends on the context in which the call is received and the behavioral responses made by the recipient of the call (Adler & Anisko, 1979; Anisko et al., 1978). Preejaculatory 22-kHz vocalizations may serve to enhance receptive behaviors in uncooperative females (Anisko et al., 1978; Brown, 1979). However, thus far only data on female behavioral antecedents (e.g. low lordosis responding, high aggression), and not female responses to the call, have been collected.

Regarding the postejaculatory 22-kHz call, it has been suggested that this call maintains the separation between the mating pair during the postejaculatory interval, while at the same time keeping the female informed about the location of the male (Barfield & Geyer, 1972). Experimental observations in large enclosures (4 ft by 4 ft) reported a positive correlation between the amount of ultrasounds emitted and the amount of spacing between the mating pair (Barfield et al., 1979). However, since males were relatively immobile while vocalizing, this effect may have been due to female avoidance of the male. Nevertheless, no study has yet to demonstrate a direct one-to-one relationship between 22-kHz vocalizations and inhibition of female approach and solicitation (Adler & Anisko, 1979 Anisko et al., 1978).

Conceivably, the postejaculatory 22-kHz call may facilitate female refractoriness (Adler & Anisko, 1979). This would be adaptive, since copulatory intromissions delivered too soon after ejaculation

can disrupt sperm transport (Adler & Zoloth, 1970). Alternatively, if the potential of mating taking place in a group setting with several males present is considered, then postejaculatory 22-kHz calls may serve to inhibit male aggression directed at postejaculatory males. Indeed, resident males directed less aggression toward vocalizing male intruders than toward silent males (Lore et al., 1976). Furthermore, Adler & Anisko (1979) recently demonstrated that resident males exhibited a longer latency to attack an intruder male which had just previously ejaculated and was emitting the 22-kHz call than an intruder which was not given any pretest sexual experience.

Golden Hamsters

Ultrasounds were found to facilitate various female behavioral responses (Floody & Pfaff, 1977b). Specifically, females exhibited a preference to approach either female or synthetic ultrasounds played through a speaker in an arm of a Y-maze. Estrous females exhibited a stronger preference for both stimuli than diestrous females. Furthermore, natural female calls were more attractive than the synthetic calls that were used to mimic male ultrasounds. Clearly, a similar study needs to be performed where female preference for natural female versus natural male vocalizations is examined. The authors suggested that females may respond to female ultrasounds because males generally are present at a site where female ultrasounds are being produced. Thus, it would be adaptive for females to approach a source of female ultrasounds. Several other experiments provided more convincing evidence of the role male ultrasounds play in facilitating female reproductive behaviors. First, estrous females

were found to increase their rate of ultrasound production after exposure to synthetic male ultrasounds (Floody & Pfaff, 1977b). Also, in the absence of males, synthetic ultrasounds activated lordosis by estrous females (Floody & Pfaff, 1977b). A similar finding on the functional significance of male ultrasounds was reported by Beach, Stern, Carmichael, & Ranson (1976). They found that deafening of estrous females eliminated the attraction and lordosis which they exhibited toward a caged male.

The responses of males to female ultrasounds has not been as extensively examined as female responses to male ultrasounds. Estrous females normally vocalize until lordosis is assumed, at which time vocalizing ceases. Playback of recorded female ultrasounds stimulated male calling (Floody et al., 1979b). Furthermore, in a Y-maze, males also exhibited a preference for the arm in which recorded female ultrasounds were played over the arm without ultrasound (Floody & Pfaff, 1977b). Apparently, female hamster ultrasounds attract conspecific males and, also, stimulate them sexually.

Other Species

The functional significance of ultrasonic communication has not been adequately investigated in other rodent species. In collared lemmings, although the responses to conspecific ultrasounds were not directly examined, there is an indication that ultrasonic vocalizations may facilitate mating. Comparisons of latencies to first contact, mount, lordosis, and ejaculation revealed that these scores tended to be shorter in tests with ultrasonic vocalizations present than in tests without ultrasounds. Obviously, much more

comparative work needs to be done on animal responses to conspecific ultrasonic signals.

Objectives of the Present Study

The preceding review presented a great deal of evidence on ultrasonic communication during sexual behavior in rodents. However, the bulk of this evidence dealt with studies on 3 different laboratory species; house mice, rats, and golden hamsters. Although the occurrence of ultrasonic vocalizations during sexual encounters has been verified in other laboratory and wild-trapped species, research on the causal mechanisms and functional significance of ultrasonic communication in these species is lacking. There is a need for more extensive research in these less commonly used rodent species, so that general principles of ultrasonic communication can be derived.

Sales (1972) noted the occurrence of ultrasonic vocalizations during courtship and copulatory behavior of deer mice (Peromyscus maniculatus). However, this report was based on only 4 observations. The present study initially sought to replicate these observations and determine whether ultrasonic vocalization behavior was sexually dimorphic. Subsequently, an attempt was made to identify causal mechanisms and adaptive functions of ultrasonic calling during deer mice mating encounters. In order to achieve these objectives, I concentrated on the same broad areas which were discussed in the literature review. To reiterate these are: (1) External or environmental stimuli which elicit ultrasonic vocalizations; (2) Behavior of the ultrasound producer and endogenous factors influencing

ultrasound production; and (3) Behavioral responses to ultrasonic vocalizations.

GENERAL METHODS

Animals.

The animals used in this experiment were male and female deermice (Peromyscus maniculatus bairdi) bred in the laboratory from stock originally trapped in East Lansing, Michigan. All were 3-4 months of age at the beginning of behavioral testing. Deer mice were housed individually in plastic cages, 48 X 27 X 13 cm for males , and 27 X 16 X 14 cm for females. Wood shavings were used for bedding and cotton Nestlets (Ancare Corp.) were provided as nesting material. Food and water were available at all times. Deer mice were maintained on a reverse day-night cycle of 16 hr light and 8 hr dark with lights off at 1030.

Apparatus.

Ultrasonic vocalizations were monitored on a QMC Instruments Ltd. Mini-Bat Detector (London) tuned to a center frequency of 35 kHz. The QMC detector transforms ultrasounds into low frequency audible signals. This device was positioned 25 cm above the floor of the male's cage. Deer mice ultrasonic vocalizations were clearly distinguishable from other ultrasounds produced by the animals (i.e., scratching, digging, and gnawing about the cage).

Tests for Ultrasonic Vocalizations During Sexual Behavior.

To induce sexual receptivity, intact females were injected subcutaneously with 60 ug estradiol benzoate approximately 72 hr before behavioral testing and 600 ug progesterone approximately 6 hr before testing. For certain experiments untreated ovariectomized females were used. Sexual behavior tests began with the introduction

of the receptive female into the male's home cage approximately 4 hr after lights off. The room in which the tests were conducted was illuminated by a 25 watt red bulb.

Male copulatory behavior in P. m. bairdi consists of mounts (with thrusting), intromissions (with penile insertion), and ejaculation. The temporal pattern of the behavior is such that after several intromissions the male ejaculates. Following ejaculation there is a period of sexual quiescence (postejaculatory interval) before sexual behavior resumes. Ultrasonic vocalizations, mounts, intromissions, and ejaculations were recorded on an Esterline Angus Event Recorder. Sexual behavior tests were terminated when one of the following criteria had been satisfied: (1) 15 min after the start of the test with no intromission; (2) no ejaculation within 15 min of the first intromission; (3) no intromission within 10 min following ejaculation; (4) first intromission following ejaculation.

Measures.

From the experimental data the following behavioral measures were derived: mount latency (ML) - time in seconds from introduction of the female to the first mount; intromission latency (IL) - time in seconds from introduction of the female to the first intromission; ejaculation latency (EL) - time in seconds from intromission to ejaculation; mount frequency (MF) - number of mounts preceding ejaculation; intromission frequency (IF) - number of intromissions preceding ejaculation; mean interintromission interval (MIII) - mean inteval in seconds between intromissions including the interval between the last intromission and ejaculation; postejaculatory interval (PEI) - time in seconds from ejaculation to the next intromission; vocalization latency (VL) - time

in seconds from introduction of the female to the first 35-kHz vocalization; postvocalization intromission latency (PVIL) - time in seconds from the production of the first 35-kHz ultrasound to first intromission; vocalization frequency (VF) - total number of 35-kHz vocalizations; vocalization rate (VR) - number of 35-kHz vocalizations per minute. VF and VR were calculated for the periods before copulation, during copulation, and after ejaculation. These three sampling periods correspond to the PVIL, EL, and PEI, respectively.

Tests for Ultrasounds with an Anesthetized Stimulus Animal.

Intact males were paired with receptive or ovariectomized females. One member of the male-female pair was selected to be anesthetized 15 min before the start of testing with an intraperitoneal injection of chloral hydrate (1mg/gm body weight) and returned to their home cage. Testing began with the introduction of the female (either awake or anesthetized) into the male's cage. The production of 35-kHz ultrasounds was monitored for 10 min.

EXPERIMENT 1

The purpose of this experiment was to investigate whether any relationship exists between ultrasonic vocalizations and sexual behavior in deer mice (Part A). Also, a determination was made of the sexual partner that produced ultrasounds during mating (Parts A, B & C).

Method

Part A - Relationship Between Sexual Behavior and Ultrasonic Vocalizations

Subjects and Procedure.

Forty-one male and 41 sexually receptive female deer mice were used in this experiment. In at least two sexual behavior tests prior to the start of the experiment, hormone treatment had successfully stimulated receptivity in these females. Also, before the experiment, males were given one 30 min unmonitored session with a receptive female. One week after this session, males and females were observed in sexual behavior tests, once per week, for two consecutive weeks. For each test the animals were paired with a different partner.

Part B - Evidence for Sexual Dimorphism of Ultrasonic Calling

Subjects and Procedure.

Subjects were 14 sexually experienced male-female pairs derived from the animals used in Part A. Deer mice pairs were tested for ultrasonic vocalizations with one member of the pair being selected to

be anesthetized, so that, in 7 pairs, the male was anesthetized and, in the other 7 pairs, the female was anesthetized. In all cases the female was was receptive.

Part C - Further Evidence for Sexual Dimorphism of Ultrasonic Calling

Subjects and Procedure.

Four sexually experienced males were paired with 4 receptive females in 15 min sexual behavior tests. On the day following this test, the inferior laryngeal nerves of each male were bilaterally sectioned under Metofane (Pitman Moore, Inc). anesthesia. One week after this surgery the same deer mice pairs were tested for ultrasounds during sexual behavior.

Results

Part A

During the first week of testing for sexual behavior (Table 2), the occurrence of male copulation was associated with the occurrence of ultrasonic vocalizations, whereas failure to copulate was associated with the absence of ultrasonic emissions ($\chi^2(1)=24.0$, $p<.001$). Further, in tests resulting in copulation, precopulatory ultrasounds were always detected. Males that vocalized and copulated during the first week of testing, continued to do so during the second week. Also, three of the four males that vocalized, but failed to copulate in the first week, copulated and emitted ultrasounds in the second week. During the second week, ultrasounds and male sexual

TABLE 2

Relationship Between Male Copulation and Ultrasonic Vocalizations

	Ultrasonic Vocalizations	
	Present	Absent
Week 1		
Number of Males Copulating	22	0
Number of Males not Copulating	4	15
Week 2		
Number of Males Copulating	25	0
Number of Males not Copulating	1	15

behavior were absent in males that did not exhibit these behaviors in the first week.

From those tests in which vocalizations were present, VL did not vary from week 1 to week 2, with the overall mean (±SEM) for both weeks of testing being 121 ± 32 sec. Additionally, overall VR was similar for weeks 1 and 2 with an overall mean (±SEM) of 3.2± 0.6. More complete analyses of vocalization and copulatory behavior data during these tests are presented in Experiment 3, Part A.

VR during weeks 1 and 2 was compared for each male and each female to determine whether one sex was more likely responsible for ultrasound production. For males, the Pearson product-moment coefficient of VR between weeks 1 and 2 was .7807 ($p<.001$). Due to the possibility that the 15 males, which failed to exhibit ultrasounds, were influencing the analysis, they were eliminated and a correlation coefficient of .6694 ($p<.001$) was obtained for the remaining 26 males. The correlation coefficient for females was not significantly different from 0 if the analysis considered all females ($r=-.0196$) or only females that were in at least one test with ultrasounds present ($r=-.1724$). These data provide initial evidence that males rather than females were producing ultrasonic calls.

Part B

Six out of seven males produced 35-kHz vocalizations when tested with anesthetized receptive females. Mean (±SEM) VF during the 10 min test was 16.1± 3.1. No ultrasounds were detected when receptive females were paired with anesthetized males. These data support the

notion that only males produced ultrasounds during sexual behavior testing.

Part C

Prior to the sectioning of the inferior nerves of males, ultrasonic vocalizations were detected during all male-female pairings. Also, copulatory behavior occurred in 3 of the 4 pairings. Following the nerve section, no ultrasounds were detected from any of the 4 pairs of deer mice. Other than the inability to vocalize, these males did not appear physically disabled; however, only 1 of 4 males successfully copulated.

Discussion

The results of this study indicate that male deer mice always produced 35-kHz ultrasonic vocalizations before the onset of copulatory behavior. Furthermore, in males failing to exhibit copulatory behavior, ultrasonic calls were seldom detected. This strong association between male copulatory behavior and ultrasonic vocalizations indicates that the 35-kHz calls may be an important component of the sexual behavior repertoire of male deer mice.

Several observations support the conclusion that males were responsible for producing the 35-kHz ultrasounds detected during mating tests. For males, VR was positively correlated across the two mating tests, whereas for females no such correlation was found. Second, males vocalized when paired with anesthetized receptive females, whereas receptive females did not produce ultrasounds when

paired with anesthetized males. Finally, and most conclusively, after male deer mice had their inferior laryngeal nerves transected, no ultrasonic vocalizations were detected from heterosexual pairs which in previous tests had exhibited ultrasounds. A similar sexual dimorphism of ultrasound behavior during mating has been reported for house mice (Sales, 1972; Whitney et al.,1973), rats (McIntosh & Barfield, 1980), and Mongolian gerbils (Holman, 1980).

EXPERIMENT 2

Experiment 1 established that male deer mice respond to sexually receptive female deer mice by producing ultrasonic vocalizations. In Experiment 2, the nature of the female cues which elicit male vocalizations was investigated. Part A contrasted the ability of females in artificially induced estrus to elicit ultrasounds against females that were ovariectomized. In Part B, an initial determination was made of the necessary and/or sufficient female stimuli that promote male deer mice ultrasonic vocalizations.

Method

Part A

Subjects and Procedure.

Subjects were 8 sexually experienced male deer mice. Eight female deer mice, 4 of which were ovariectomized (OVX) and 4 of which were sexually receptive, served as stimulus animals. Males were observed for ultrasounds during sexual behavior tests, once per week, for two consecutive weeks. The order of presentation of the two stimulus conditions was counterbalanced so that males were tested with a sexually receptive female one week and an OVX female on the other week.

Part B

Subjects and Procedure.

Subjects were 12 sexually experienced male deer mice. Stimulus females were the same as those used in Part A. Six of the males were randomly selected to be paired with an anesthetized receptive female, and the remaining 6 males were paired with an anesthetized ovariectomized (OVX) female. Ultrasonic vocalizations were monitored for 10 min from males paired with anesthetized females, either receptive or OVX.

Results

Part A

The percentage of males exhibiting ultrasounds was significantly higher when males were paired with behaviorally receptive females than when males were paired with OVX females (Fisher's exact probability test, $p<.05$). Whereas all 8 males vocalized with receptive females, only 2 out of 8 males vocalized with OVX females. Mean VF of the two males which vocalized with OVX females was 15.5, as compared to a mean VF of 34.0 when these same two males were paired with receptive females. Finally, it should be noted that 7 out of the 8 males copulated and exhibited precopulatory ultrasonic vocalizations during their tests with receptive females.

Part B

No males produced ultrasounds when presented with an anesthetized OVX female. In contrast, similar to the results of Exp. 1, Part B, 5

out of 6 males vocalized when paired with an anesthetized receptive female. Mean (±SEM) VF of these males was 13.6 ± 3.2, comparable to the results reported in Exp. 1, Part B.

Discussion

The results of these experiments demonstrated that male deer mice respond to sexual signals of conspecific females with ultrasonic vocalizations. Part A established that for most males, cues associated with a sexually receptive female were necessary for eliciting male ultrasounds, since males generally produced ultrasonic vocalizations only when females were sexually receptive. Ultrasound tests with anesthetized females (Part B) also confirmed this finding. Males only vocalized with receptive females and not with OVX females. Moreover, these findings were suggestive of the posssibility that chemical signals produced by receptive females were sufficient to promote male ultrasound responding.

The experimental findings using awake females as stimulus animals differed in several respects from those reported in similar experiments with other species. Male house mice did not exhibit differential rates of ultrasound responding to females in different ovarian hormone states (Nyby et al., 1981). In both laboratory rats and hamsters, males continued to vocalize to OVX females or diestrous females, but not at as high a rate as when tested with females in estrus (Floody et al., 1977; Geyer & Barfield, 1978). This difference in the male's response to females in different ovarian hormone states was quantitative rather than qualitative. In contrast to these

species, most male deer mice did not vocalize with OVX females.

In other rodent species (Nyby & Whitney, 1978), olfactory cues produced by female deer mice may be important for eliciting male calling. The manner in which these chemosignals were transmitted by receptive females and sensed by males needs to be more fully explored. Additionally, it is important to stress that although chemosignals were sufficient for eliciting male deer mice ultrasounds, sexually active females likely performed other behaviors which also facilitated male vocalizations. This suggestion is supported by the observation that male deer mice vocalized at a higher rate when paired with an awake female than when paired with anesthetized receptive females. Similar results confirming the influence of female activity on male ultrasound were also reported in laboratory rats and hamsters (Floody et al., 1977; Geyer & Barfield, 1978).

EXPERIMENT 3

The preceding experiment examined external stimuli that are involved in triggering male deer mice ultrasonic vocalizations. In this experiment, the behavior of the vocalizing male was considered. By observing the male's behavior, additional clues concerning the proximate causes of ultrasonic signalling and the information contained in the ultrasonic message might be provided. Part A of this experiment explored the temporal relationships that exist between male ultrasounds and male copulatory behavior by subjecting the data collected in Exp. 1 to further analysis. In Part B, the relationship of male ultrasounds and copulatory behavior to other male behaviors was examined.

Method

Part A

Subjects and Procedure.

Subjects and procedure used were discussed in Exp. 1, Part A. Data originally collected in that experiment were analyzed.

Part B

Subjects and Procedure.

Subjects in this experiment were 47 male deer mice. Males were given a 30 min unmonitored session in their home cage with a receptive female. One week after this session, males were observed for ultrasounds during a sexual behavior test. Sexually receptive females

were used as stimulus animals. The following male behaviors were recorded in addition to those outlined in the General Methods: male solicitation - male approach toward female from greater than 1 body length to less than 1 body length; and male locomotor activity - amount of time in seconds, male ambulating or running about the cage (if the male was motionless for less than one second during a locomotor sequence, the activity was judged continuous).

Measures.

In addition to the measures described in the General Methods the following measures were derived from the behavioral data: male solicitation frequency - total number of male solicitations; male solicitation rate - number of male solicitations per min; and % male locomotor activity - % of time in which male involved in locomotor activity.

Results

Part A

From tests in which males copulated to an intromission following ejaculation, comparisons of the VF and VR before copulation, during copulation, and after ejaculation were made (during the PVIL, EL, and PEI, respectively). There were no significant differences in ultrasound performance between weeks 1 and 2; therefore, the data for each male over the two weeks of testing were averaged with the means (±SEM) shown in Figure 1. VF varied during the three time periods (one-way ANOVA, $F[2,26]=13.4$, $p<.01$). Planned comparisons revealed that VF was higher after ejaculation than before copulation

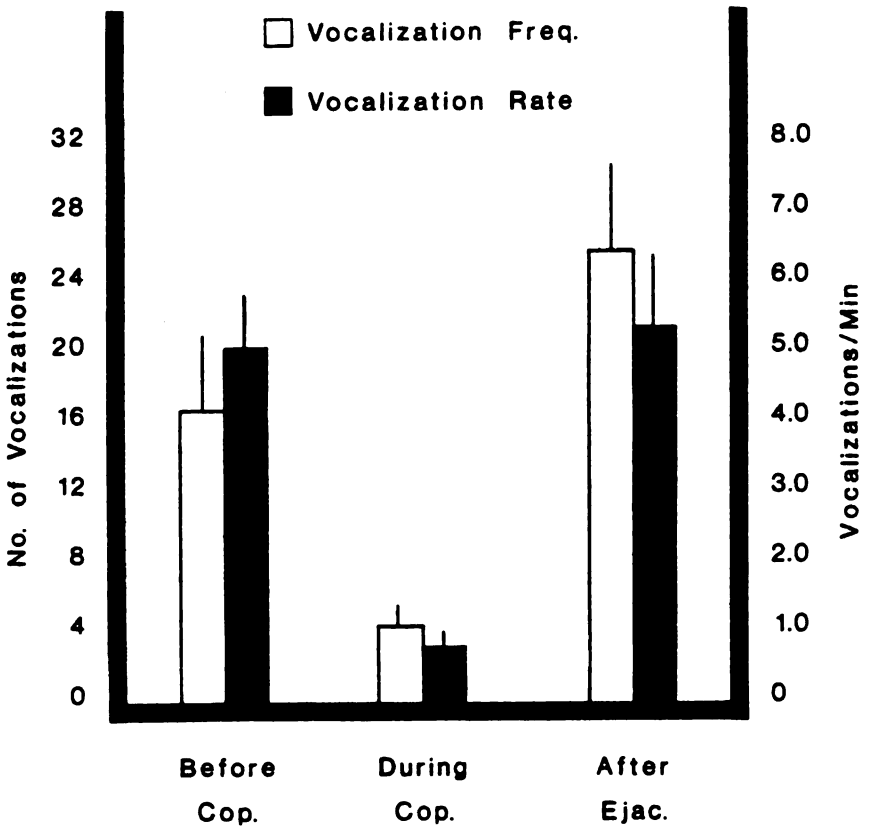

Figure 1. Mean (±SEM) vocalization frequency and vocalization rate of male deer mice before copulation (PVIL), during copulation (EL), and after ejaculation (PEI).

($F[1,26]=4.95$, $p<.05$), and that both of these time periods had a significantly higher VF than that found during copulation ($F[1,26]=27.0$, $p<.01$). VR also varied during the three time periods (one-way ANOVA, $F[2,26]=17.0$, $p<.01$). Again, VR before copulation and after ejaculation was significantly higher than VR during copulation ($F[1,26]=33.8$, $p<.01$). Moreover, precopulatory and postejaculatory VR did not differ; indicating that the differences observed in VF during these two time periods was simply a result of the postejaculatory time span being longer than the precopulatory time period. It should also be noted that ultrasonic calls monitored during the copulatory period were detected during the intervals between mounts and intromissions and, occasionally, were synchronous with ejaculation, but were never detected during a mount or an intromission.

Relationships between measures of male copulatory behavior and ultrasonic vocalizations were evaluated by performing Pearson product-moment correlations. Independent correlation coefficients were computed using data from the first and second test in which both sex behavior and ultrasounds were exhibited. A significant negative correlation existed between VR before copulation and the length of the PVIL for both the first ($n=25$, $r=-.4775$, $p<.01$) and second test ($n=22$, $r=-.3661$, $p<.05$). VL was not found to be related to precopulatory VR or to any measures of sex behavior performance. In the males' first test with ejaculation, VR during copulation was not correlated with measures of male sexual performance (i.e. MF, IF, EL, and MIII). However, in the males' second test with ejaculation, there was a highly positive correlation between VR during copulation and the length of the MIII ($n=20$; $r=.6694$, $p<.01$), but no relation with other

measures of sexual performance. Finally, VR after ejaculation was significantly and positively correlated with the length of the PEI in the males' first test ($n=8$, $r=.6653$, $p<.05$), but not in their second test.

Part B

On the basis of the sex behavior tests, males could be classified into 3 independent groups: (1) males exhibiting ultrasounds and copulatory behavior ($N=15$); (2) males exhibiting ultrasounds but no copulatory behavior ($N=16$); and (3) males not exhibiting either ultrasounds or copulatory behavior ($N=16$). Of the two groups of males producing ultrasonic vocalizations, copulating males had a significantly higher VR than non-copulating males ($t(29)=3.67$, $p<.01$). More importantly, precopulatory VR of copulating males was significantly higher than the overall VR of vocalizing, non-copulating males ($t(29)=9.76$, $p<.01$), with the means ($\pm$SEM) being $3.3 \pm .4$ and $1.3 \pm .2$, respectively.

Solicitation frequency, solicitation rate, and locomotor activity of the 3 groups of males is presented in Table 3. Solicitation frequency and solicitation rate varied across the 3 groups such that the two groups of non-copulating males did not differ, but both groups had a significantly lower solicitation frequency and rate than vocalizing and copulating males. The 3 male groups did not differ in locomotor activity.

Solicitation rate and locomotor activity during the VL in both groups of vocalizing males were similar to the overall solicitation rate and locomotor activity observed in non-vocalizing and

TABLE 3

COMPARISON OF MALE SOLICITATION FREQUENCY, SOLICITATION RATE, AND LOCOMOTOR ACTIVITY IN 3 GROUPS OF MALE DEER MICE

	VOCALIZING & COPULATING MALES	VOCALIZING & NON-COPULATING MALES	NON-VOCALIZING & NON-COPULATING MALES
MALE BEHAVIOR[a]			
Solicitation frequency	41.2 ± 5.0	16.8 ± 2.2[b]	21.4 ± 5.5[b]
during VL	4.7 ± 1.4	4.2 ± .8	
after VL	36.5 ± 6.3	12.7 ± 1.9	
Solicitation Rate	3.0 ± .4	1.1 ± .1[b]	1.4 ± .4[b]
during VL	1.2 ± .4	1.2 ± .2	
after VL	3.3 ± .4	1.1 ± .2	
% Locomotor Activity	89.6 ± 3.1	85.2 ± 4.1	81.4 ± 6.5
during VL	87.2 ± 2.9	81.9 ± 6.6	
after VL	90.4 ± 3.8	89.3 ± 3.6	

[a] for all measures mean (± SEM)
[b] $p < .05$ vs copulating & vocalizing males, one-way ANOVA followed by Student-Newman-Keuls procedure

non-copulating males. In males that vocalized and copulated, solicitation rate was significantly higher after the VL than during the VL (Sign test, $p<.01$). In contrast, in males that vocalized but did not copulate, solicitation rate did not increase following the first vocalization. Locomotor activity did not change during the period following the first vocalization in either group of vocalizing males.

As shown in Figure 2, in vocalizing, copulating males, solicitation rate and locomotor activity increased significantly during the PVIL over the levels observed during the VL (Sign tests, $p<.01$ and $p<.05$, respectively). During the EL, solicitation frequency was significantly higher than during the PVIL (Sign test, $p<.01$); however, since the solicitaion rates did not differ between these two time periods, the difference observed in solicitation frequency reflected the fact that the EL time span was longer than the PVIL. Following ejaculation, both solicitation rate and locomotor activity declined significantly from the levels observed during the EL (Sign tests, $p<.001$ and $p<.001$, respectively) to a level comparable to that observed during the VL. With respect to solicitations during the PEI, it should be noted that males clustered most of their solicitations into the period immediately preceding the reinitiation of copulation (last 10% of the PEI).

Discussion

Ultrasonic signalling in male deer mice in response to the presentation of receptive females appeared to set in motion a sequence

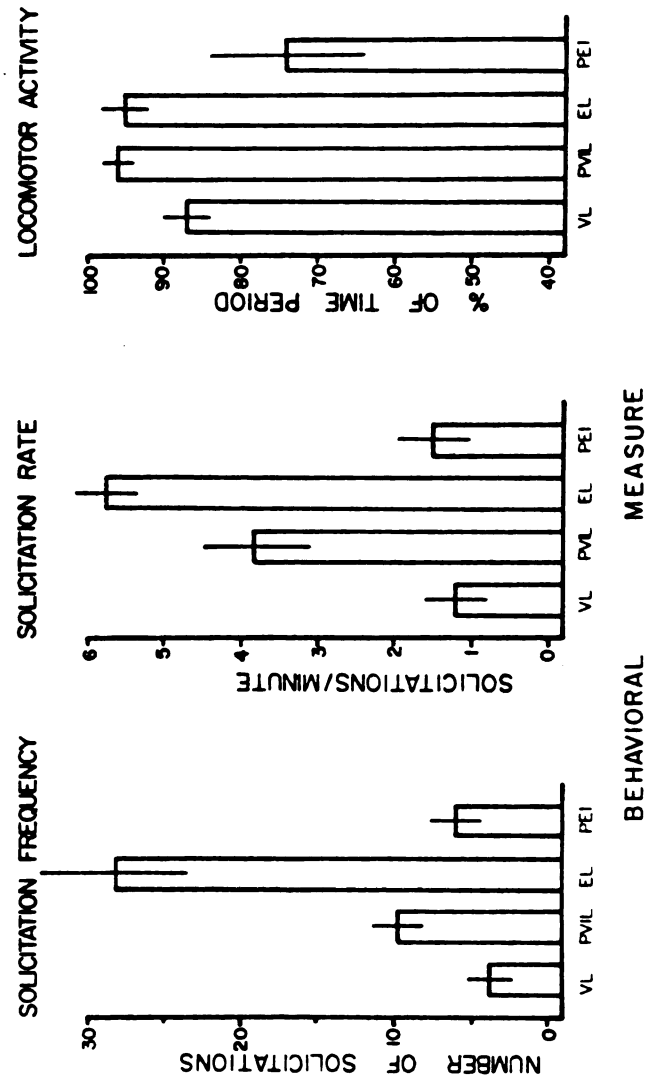

Figure 2. Mean (±SEM) solicitation frequency, solicitation rate, and % locomotor activity by vocalizing and copulating male deer mice during different temporal periods of the sexual behavior test.
Note. VL=vocalization latency, PVIL=postvocalization intromission latency, EL=ejaculation latency, and PEI=postejaculatory interval.

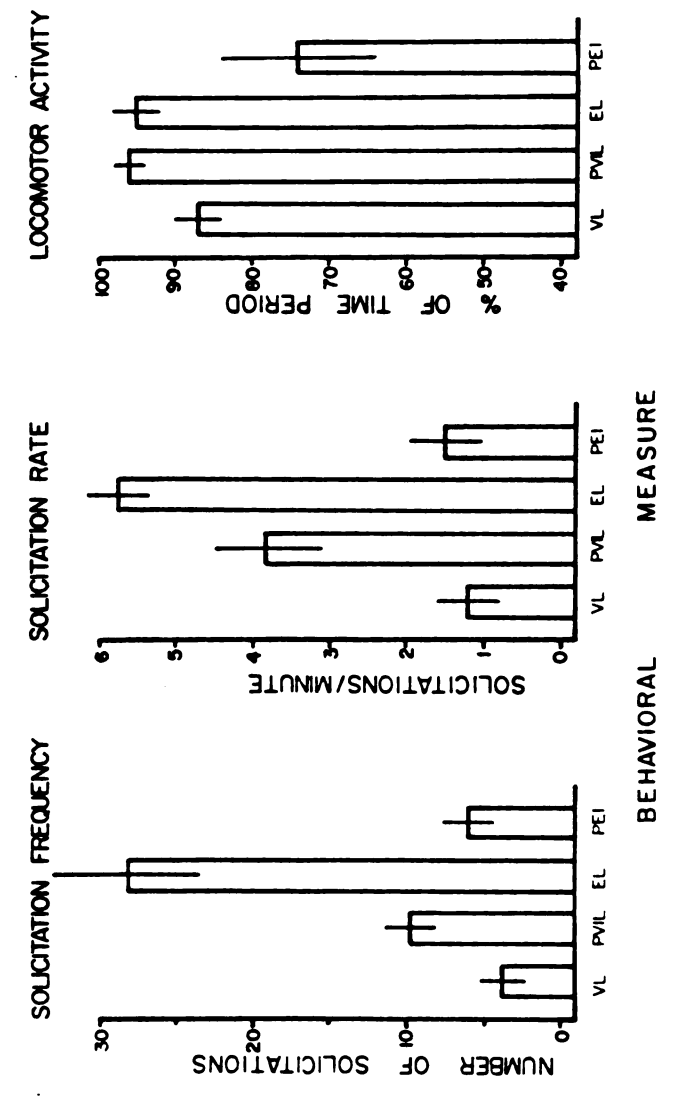

Figure 2. Mean (±SEM) solicitation frequency, solicitation rate, and % locomotor activity by vocalizing and copulating male deer mice during different temporal periods of the sexual behavior test.
Note. VL=vocalization latency, PVIL=postvocalization intromission latency, EL=ejaculation latency, and PEI=postejaculatory interval.

of events which, if performed appropriately, led to reproduction. Male behavior among copulating males did not differ from that exhibited by non-copulating males until the production of the first ultrasound. Once they began vocalizing, males that copulated increased both their rate of soliciting females and their level of locomotor activity. In contrast, non-copulating males (both vocalizing and non-vocalizing) maintained a constant low rate of solicitations and low level of locomotor activity throughout the testing session. Additionally, the VR of vocalizing, non-copulating males was much lower than the precopulatory VR observed in copulating males. In sum, the results of this experiment indicated that the initiation of copulation in deer mice is associated with high levels of male courtship ultrasonic vocalizations, solicitations, and male locomotor activity.

The temporal patterning of ultrasounds in male deer mice was such that VR was highest before the onset of copulation and after ejaculation. In comparison to these two time periods a dramatic reduction in VR was observed during copulation. Sales (1972) also reported ultrasonic emission during both precopulatory and copulatory sequences, but did not measure VR during these two time periods.

To further elucidate the relation between ultrasonic vocalizations and copulatory behavior, correlations based on performance of these two behaviors were considered. Several factors could account for the inverse relationship found between precopulatory VR and the length of the PVIL. First, precopulatory VR may indicate the male's readiness to mate. Second, precopulatory ultrasonic calls may serve a courtship function. High VR by the male could then

facilitate the onset of copulation by promoting increased levels of female proceptive and receptive behaviors (Beach et al, 1976; Floody and Pfaff, 1977b; Geyer et al., 1978b; McIntosh et al.,

Following the onset of copulation the VR declined. This decline seen in VR while the animals were copulating might indicate that during this period, vocalizations were less critical to the maintenance of female proceptive and receptive behaviors (Beach et al., 1976; Floody & Pfaff, 1977b). Additionally, along with the males' copulatory behavior, their high solicitation rate and high level of locomotor activity most likely served to sustain female behavior. It should also be noted that a positive correlation between VR during copulation and the length of the MIII was found in one of the two tests in Part A. Since this relationship was not found in both tests it may be spurious, reflecting the number of correlations that were computed. However, McIntosh and Barfield (1980) reported a similar positive correlation between VR during copulation and MIII in rats. Possibly, in males exhibiting long intervals between intromissions, it is necessary to make use of the same strategies during copulation, such as high rate of ultrasonic vocalizations, that were employed during the courtship sequence before copulation.

Male deer mice returned to high rates of vocalization following ejaculation. Also, after ejaculation, males exhibited a reduction in solicitation rate and locomotor activity similar to that found in laboratory rats (Adler & Anisko, 1979; Dewsbury, 1967). Thus, the high rate of postejaculatory calling may serve to maintain the proximity and sexual activity of the female (Barfield & Geyer, 1975), so that additional copulatory series and ejaculations can be achieved.

Female deer mice were found to require more than one ejaculation for maximal pregnancy initiation (Dewsbury, 1979); therefore, it would clearly be adaptive for males to perform behaviors which maintain the copulatory readiness of females. In rats, the 22-kHz postejaculatory call of males may inhibit potentially aggressive behavior of conspecifics (Anisko et al., 1978; Barfield & Geyer, 1975). Such a function for postejaculatory vocalizations is not incompatible with the one being proposed for deer mice.

EXPERIMENT 4

The previous experiments established that ultrasonic vocalizations by male deer mice are intimately related to other aspects of male sexual behavior. Since endogenous factors such as gonadal hormones have a major controlling influence on male deer mice copulatory behavior (Clemens & Pomerantz, 1981), it would be expected that the same sex hormones controlling copulation would also exert a similar influence on male ultrasonic calling. For cells within the central nervous system and genital tissues, it has been demonstrated that metabolism of testosterone (T) by aromatase enzymes (aromatization) leads to the production of estradiol (E) (Callard, Petro, Ryan, 1978; Lieberburg & McEwen, 1975; Naftolin, Ryan, & Petro, 1972) and T metabolism by 5α-reductase enzymes (5α-reduction) results in the production of 5α-dihydrotestosterone (DHT) and other 5α-reduced androgens (Bruchovsky & Wilson, 1968; Massa, Justo, & Martini, 1975; Whalen & Rezek, 1972). Both 5α-reduction and aromatization of T were found to be obligatory for T to reliably stimulate male copulatory behavior in castrated male deer mice (Clemens & Pomerantz, 1982). The present experiment was designed to test the extent to which T, and two of its major metabolites, E and DHT, influence male ultrasonic vocalizations.

Method

Subjects and Procedure

Male deer mice that exhibited ultrasounds and copulatory behavior in previous experiments were selected for use in the present experiment. Following their successful sexual behavior test, 72 males were castrated under Metofane anesthesia. Six weeks after castration, males were pretested for sexual behavior with a receptive female. Males were matched according to whether they exhibited ultrasounds in this pretest and were assigned to one of several different daily hormone treatment groups. Hormone treatment began the day after the pretest and continued for two weeks. In Part A, the daily treatments used were: 1 ug estradiol benzoate (EB, N=8); 2 ug EB (N=9); 3 ug EB (N=7); and sesame oil (OIL, N=8). In Part B, the daily treatments were: 50 ug dihydrotestosterone propionate (DHTP, N=8); 100 ug DHTP (N=8); and 200 ug DHTP (N=8). In Part C, the daily treatments were: 1 ug EB + 50 ug DHTP (N=8) and 200 ug TP (N=8). Hormone treatments were dissolved in .02 ml sesame oil and injected subcutaneously. Males received two sexual behavior tests with a receptive female, one per week, beginning one week after the start of hormone treatment. During the course of the experiment, one male receiving 50 ug DHTP/day and one male receiving 200 ug DHTP/day died.

Measures

All measures were the same as in the General Methods, with the exexception that overall VR was defined as the number of 35-kHz vocalizations/min starting with the production of the first ultrasound. This redifined measure was used so that VR in

non-copulating males could be compared to the precopulatory VR of copulating males.

Results

Part A

Percentages of castrated males exhibiting ultrasonic vocalizations and mounting are represented in Figure 3. On the first week, 1 ug EB EB/day activated male ultrasound production to a greater extent than any other treatment ($\chi^2(1)=4.85$, $p<.05$). By the second week, calling was stimulated to a greater extent in castrated males receiving 1 ug EB/day or 2 ug EB/day than in males receiving either 3 ug EB/day or OIL ($\chi^2(1)=13.69$, $p<.01$). It should be noted that males receiving 3 ug/day were noticeably lethargic during testing and non-testing periods.

Regarding measures of ultrasound performance, vocalization latencies of males receiving 1 ug EB/day and 2 ug EB/day were similar, with a mean (±SEM) of 416 ± 95 sec and 598 ± 106 sec, respectively. Similarly, vocalization rates of the two groups did not differ, with the mean (±SEM) of the 1 ug EB/day group being 1.0 ± .4 and the mean (±SEM) of the 2 ug EB/day group being 1.1 ± .5. Measures of ultrasound performance were not analyzed in males receiving 3 ug EB/day or OIL, since differences from the other groups in performance would only reflect differences in the percentage of males vocalizing.

Although a few EB-treated animals exhibited mounting behavior (most notably males receiving 2 ug EB/day), none of the EB treatment

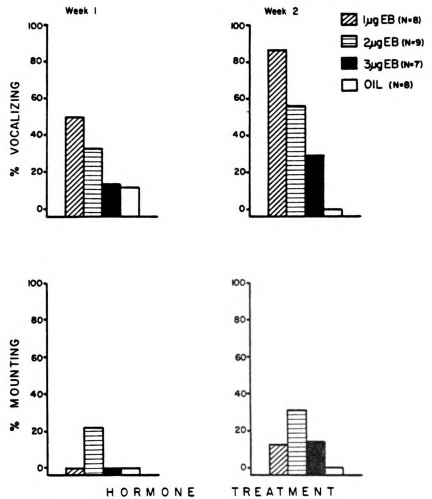

Figure 3. Percentage of castrated male deer mice exhibiting ultrasonic vocalizations and mounting behavior following treatment with 1 ug EB/day, 2 ug EB/day, 3 ug EB/day, or OIL.

groups were significantly different from OIL controls (Figure 3). Also, no males exhibited intromission or ejaculation.

Part B

Figure 4 illustrates the effects of DHTP treatment on male ultrasonic calling and male copulatory behavior. Doses of 100 ug DHTP/day and 200 ug DHTP/day restored ultrasounds in all males. The 200 ug DHTP/day dosage activated ultrasounds after only one week of treatment, whereas two weeks were necessary for the 100 ug DHTP/day dosage to be completely effective. DHTP also activated male copulatory behavior in a dose-dependent manner (Chi-square test for number of males mounting, $\chi^2(2)=7.14$ $p<.05$). Chi-square tests for the number of males in different groups exhibiting intromission and ejaculation were not significant; however, intromission frequency of males receiving 200 ug DHTP/day was significantly higher than males receiving 100 ug DHTP/day (Mann-Whitney U test, $U=9$, $p<.05$).

Comparison of measures of ultrasound performance revealed that males receiving 200 ug DHTP/day had a significantly shorter VL ($U=3$, $p<.001$) and higher VR ($U=2$, $p<.001$) than males receiving 100 ug DHTP/day. Mean (±SEM) VL of the two groups was 57 ± 16 sec and 414 ± 91 sec, respectively; and mean (±SEM) VR of the two groups was $4.4 \pm .2$ and $1.5 \pm .3$, respectively.

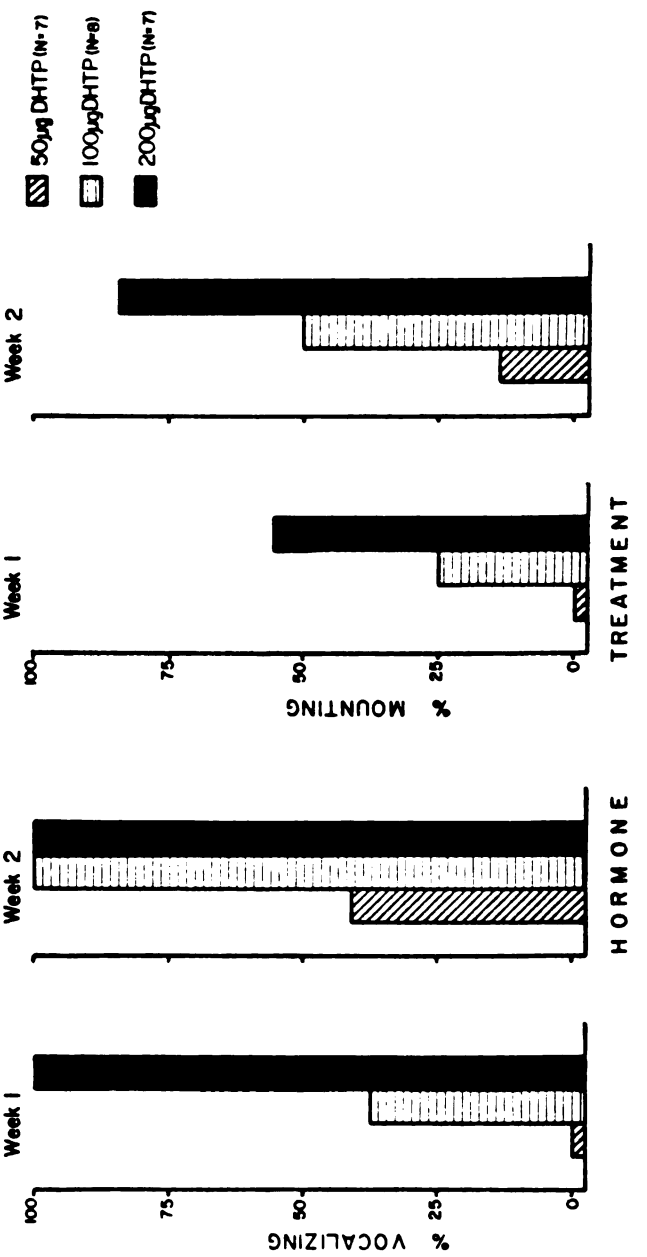

Figure 4. Percentage of castrated male deer mice exhibiting ultrasonic vocalizations, mounting, intromission, and ejaculation following treatment with 50 ug DHTP/day, 100 ug DHTP/day, or 200 ug DHTP/day.

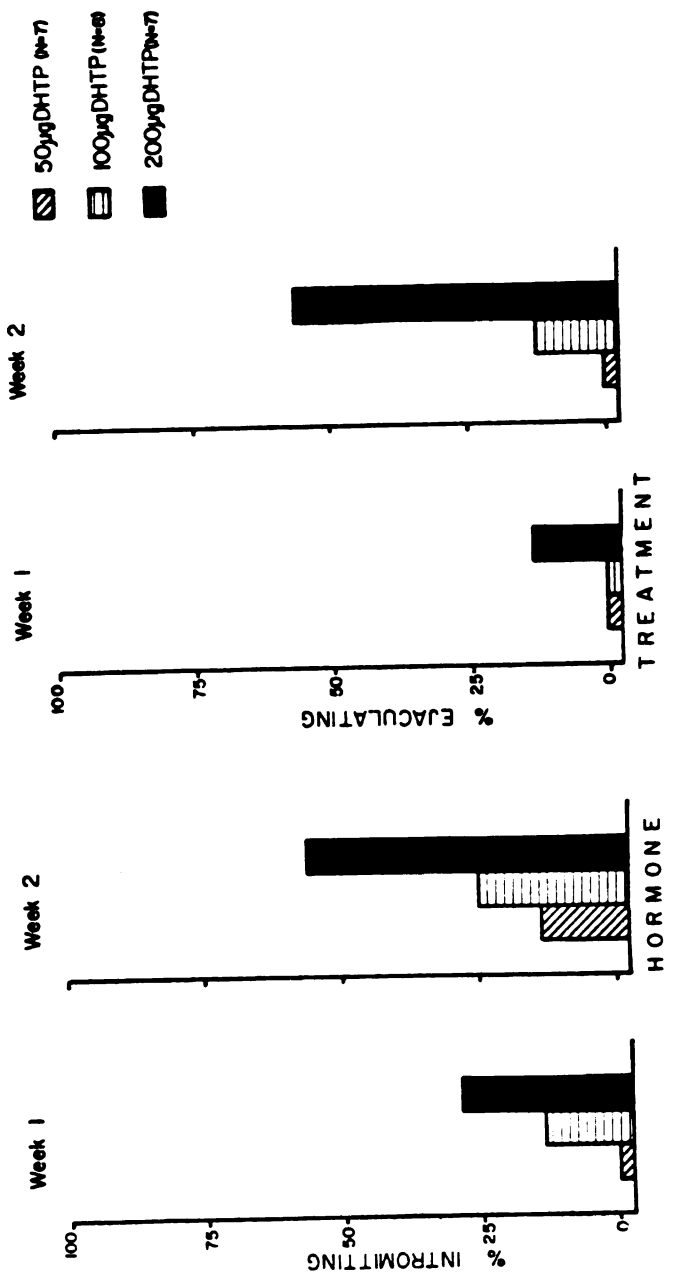

Figure 4 (cont)

Part C

Males treated with 1 ug EB/day from Part A and males treated with 50 ug EB/day from Part B were included in the analyses for this experiment. Figure 5 shows the percentages of castrated males exhibiting ultrasonic vocalizations, mounting, intromission, and ejaculation. Only males receiving 50 ug DHTP/day did not vocalize in week 1. However, by week 2, the percentage of males exhibiting ultrasonic vocalizations did not vary across treatments. Regarding copulatory behavior, combined treatment with 1 ug EB + 50 ug DHTP/day was as effective as 200 ug TP/day; and both of these treatments were significantly more effective than 1 ug EB/day or 50 ug DHTP/day (for mounting, $\chi^2(1)=7.96$, $p<.01$; for intromission, $\chi^2(1)=8.65$, $p<.01$; for ejaculation, $\chi^2(1)=9.79$, $p<.01$).

Measures of vocalization performance in the different treatment groups are compared in Table 4. VL of males receiving combined treatment of EB + DHTP was significantly shorter than males receiving TP, EB, or DHTP alone. Overall VR of TP and EB + DHTP groups did not differ; however, overall VR of the EB +DHTP group was significantly higher than males receiving DHTP alone and approached significance when compared to males receiving EB alone ($U=17$, $p<.065$). The failure of the latter comparison to reach significance could be a result of the fact that males receiving EB + DHTP did not vocalize at a high rate during copulation. Moreover, VR of the EB + DHTP group during the PVIL was significantly higher than the overall VR of males receiving EB alone ($U=6$, $p<.01$). Finally, it should be noted that males treated with TP or EB + DHTP performed similarly to intact

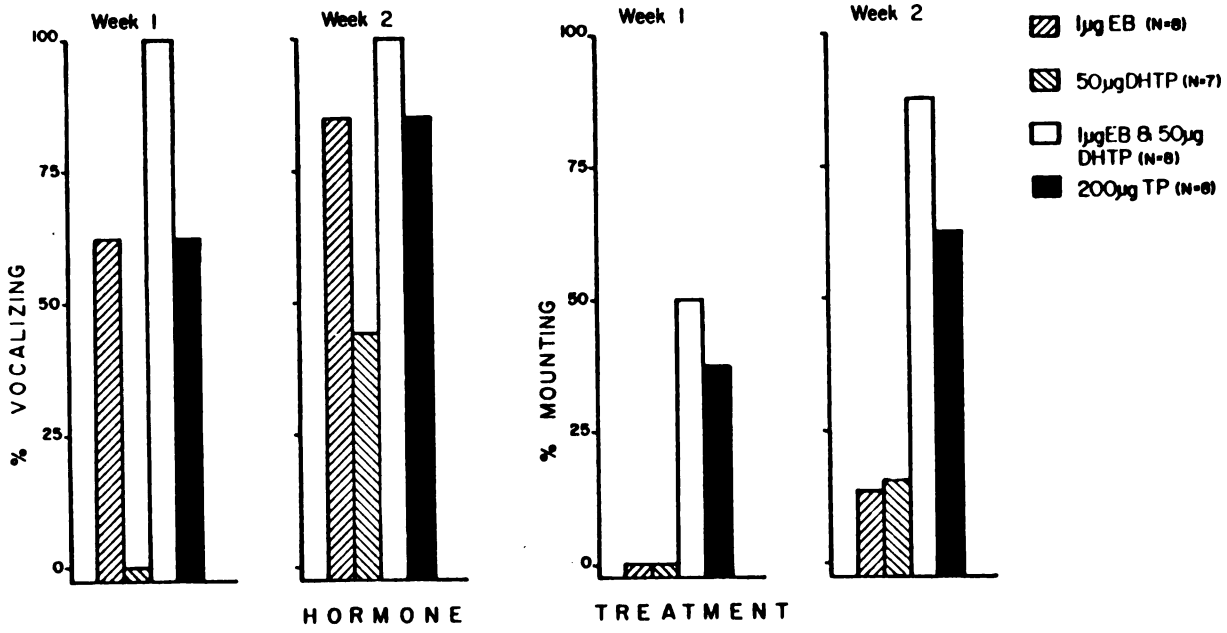

Figure 5. Percentage of castrated male deer mice exhibiting ultrasonic vocalizations, mounting, intromission, and ejaculation following treatment with 1 ug EB/day, 50 ug DHTP/day, 1 ug EB + 50 ug DHTP/day, or 200 ug TP/day.

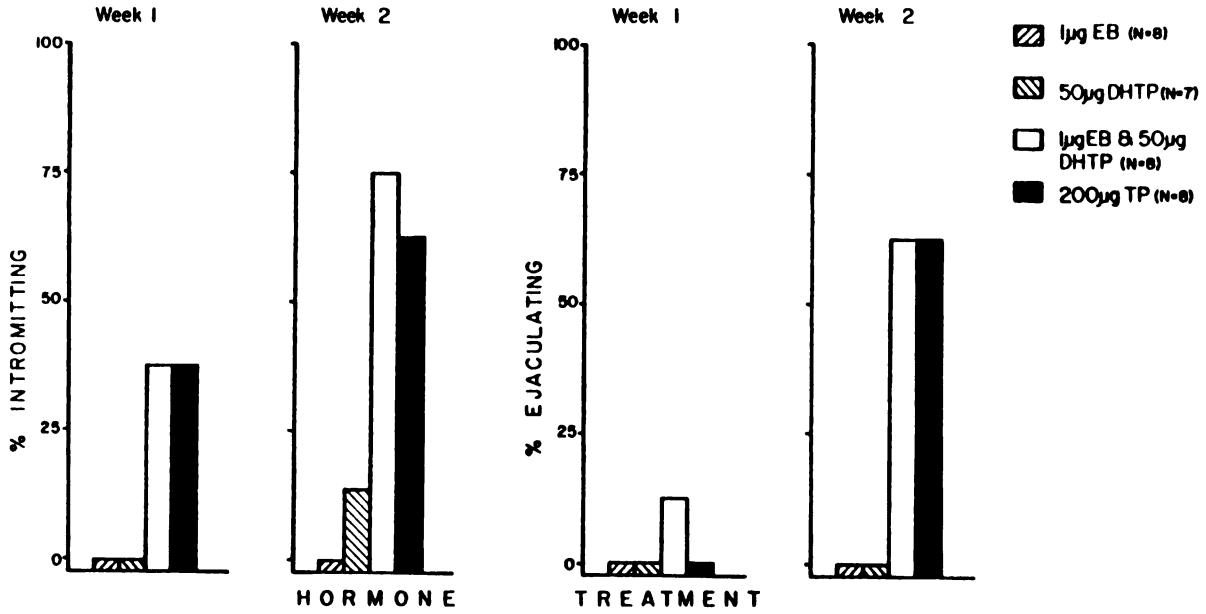

Figure 5 (cont).

TABLE 4

VOCALIZATION PERFORMANCE OF CASTRATED MALE DEER MICE GIVEN VARIOUS DAILY HORMONE TREATMENTS

	1 ug EB	50 ug DHTP	1 ug EB + 50 ug DHTP	200 ug TP
MEASURES [a]				
VL (sec)	415 ± 95 [b]	739 ± 77 [c]	182 ± 37	436 ± 91 [b]
VR (overall)	1.0 ± 0.4	0.4 ± 0.2 [c]	1.6 ± 0.2	2.6 ± 1.0
VR during PVIL	-----	-----	3.1 ± 0.6	2.9 ± 0.7
VR during EL	-----	-----	0.9 ± 0.4	1.8 ± 0.5
VR during PEI	-----	-----	3.2 ± 0.5	5.0 ± 1.8
N	8	7	8	8

[a] for all measures mean (± SEM)
[b] $p < .05$, two-tailed Mann-Whitney U test
[c] $p < .01$, two-tailed Mann-Whitney U test

males, in that their VR was lower during the EL than during the PVIL and PEI (Kruskall Wallis one-way ANOVA, $H=42.6$ $p<.001$).

Discussion

Results of this experiment demonstrated that TP, as well as two of its major metabolites, DHTP and EB, restored the performance of ultrasonic vocalizations in long-term castrated male deer mice. Additionally, both TP and its reduced metabolite, DHTP, were able to restore the complete copulatory pattern. In contrast, the aromatized metabolite of TP, EB, was only minimally effective in restoring mounting behavior. Finally, evidence was obtained which indicates that aromatized and reduced metabolites of T may synergize to control male sexual behavior in this species.

As indicated previously (Clemens & Pomerantz, 1981), TP and DHTP were equipotent in facilitating male sexual behavior. These results lead to the possibility that 5α-reduction of TP to DHTP may contribute to the activation of male sexual behavior in deer mice. Such a notion was supported by the finding that TP activation of male copulatory behavior in castrated male deer mice was inhibited by simultaneous administration of a compound that blocked 5α-reduction (Clemens & Pomerantz, 1982).

Despite the production of ultrasonic vocalizations by castrated males receiving EB, replication of the facilitation of mounting behavior by EB was not seen (Clemens & Pomerantz, 1981). There were several differences in the methodology used in the present study which may account for the failure of EB to restore mounting behavior.

First, in the earlier Clemens & Pomerantz study, 3 weeks of EB treatment was required for a significant percentage of castrated males to mount females. In the present experiment, castrated males were only treated for two weeks. Secondly, the mean mount latency of EB-treated males in the Clemens & Pomerantz study was longer than the 15 min test used in the present experiment. Nevertheless, in the present experiment, EB did stimulate vocalizations and did synergize with a subthreshold dosage of DHTP to activate male sexual behavior. These results stongly suggest that E does play a significant role in mediating male sexual behavior of deer mice. This hypothesis is further supported by the finding that TP activation of male copulatory behavior in castrated males was prevented by simultaneous administration of an aromatization inhibitor (Clemens & Pomerantz, 1982).

Although a significant percentage of castrated males receiving 1 ug EB/day exhibited ultrasounds and a small percentage of castrated males receiving 50 ug DHTP/day vocalized, males in both of these groups did not vocalize as soon after the introduction of the female, or at as high a rate as males receiving a combined dosage of both of these hormones. The synergistic action of EB and DHTP on male sexual behavior supports the notion that in deer mice, T activates male reproductive behavior by being metabolized to 5α-reduced androgens and estrogens. That is to say, T may be acting as a prohormone (Clemens & Pomerantz, 1982).

In all species thus far investigated, T has been found to activate male ultrasonic vocalizations (Floody et al., 1979; Nunez et al., 1978; Parrott & Barfield, 1975). Similar to deer mice, E

facilitated male ultrasonic vocalizations in house mice (Nunez et al., 1978) and hamsters (Floody, 1981). In contrast to deer mice, DHT did not influence ultrasound production in either of these species. Generally, species differences and similarities in the abilty of DHT or E to mediate ultrasonic vocalizations were also observed in the ability of these hormones to mediate copulatory behavior (Floody, 1981; Luttge, 1980). It remains to be determined whether species differences in the ability of metabolites of T to activate male sexual behavior reflect varying degrees of reliance on T metabolism by 5α-reduction and/or aromatization.

EXPERIMENT 5

The functional significance of a particular behavior is often analyzed by observing the consequences of performing that behavior. In the previous experiments it was suggested that ultrasound production by male deer mice may be involved in and possibly facilitate copulation by a mating pair. By observing the consequences of male vocalizations on female behavior, the final experiment was designed as an initial attempt to address the fuctional significance of ultrasonic vocalizations. Beach (1976) coined the term "proceptive behavior" for female "appetitive responses evoked by stimuli from males which initiate or increase the probability of masculine sexual behavior directed at the female." (p.116). If, as previously suggested, ultrasonic vocalizations serve a courtship function, then male ultrasounds should facilitate female proceptive behavior.

Method

Subjects and Procedure

Subjects in this experiment were 55 sexually receptive female deer mice. For all females, hormone treatment stimulated behavioral receptivity in two consecutive, weekly, sex behavior tests conducted prior to the start of the experiment. Two groups of stimulus males were derived from behavior testing conducted before the start of the experiment. One group consisted of males which copulated during a 30 min. session with a receptive female (N=30), while the other group included males that did not copulate during two consecutive, weekly,

30 min sessions (N=25). In the experiment, females were observed during sexual behavior tests with males from one of the two stimulus male groups. In addition to those behaviors outlined in General Methods, the following female proceptive behaviors were recorded: (1) female solicitation - female approach toward the male from greater than 1 body length to less than 1 body length; (2) female locomotor activity - amount of time in seconds female spent ambulating or running about the cage (Note 2); and (3) proximity - amount of time in seconds male and female within 1 body length of each other (Note 3).

Measures

In addition to the measures described in General Methods, the following measures were derived from the behavioral data: female solicitation frequency - total number of female solicitations; female solicitation rate - number of female solicitations per min; % female locomotor activity - % of time in which female involved in locomotor activity; and % proximity - % of time in which proximity was maintained between the male and female.

Results

Females were classified as experiencing 1 of 3 experimental conditions depending on the behavior of the male with which they were tested. These experimental conditions were: (1) males exhibiting ultrasounds and copulatory behavior (N=22); (2) males exhibiting ultrasounds but no copulatory behavior (N=16); and (3) males exhibiting neither ultrasounds nor copulatory behavior (N=17). As shown in Table 5, females tested with males that vocalized, regardless

TABLE 5

INFLUENCE OF MALE BEHAVIOR ON FEMALE PROCEPTIVE BEHAVIOR IN DEER MICE

	MALE STIMULUS CONDITIONS		
	VOCALIZING & COPULATING MALES	VOCALIZING & NON-COPULATING MALES	NON-VOCALIZING & NON-COPULATING MALES
FEMALE PROCEPTIVE BEHAVIOR[a]			
Solicitation frequency	5.8 ± 1.5	5.9 ± 1.7	2.2 ± 1.2[b]
during VL	0.6 ± 0.4	0.6 ± 0.3	
after VL	5.2 ± 1.8[d]	5.3 ± 1.9[d]	
Solicitation rate	.48 ± .20	.41 ± .13	.15 ± .05[b]
during VL	.41 ± .17	.34 ± .08	
after VL	.52 ± .21	.44 ± .20	
% Locomotor activity	81.1 ± 3.5	73.6 ± 4.9	53.8 ± 6.2[b]
during VL	64.3 ± 7.0	54.3 ± 7.5	
after VL	85.4 ± 4.2[c]	78.9 ± 4.0[c]	
% Proximity	21.8 ± 3.0	13.4 ± 3.1	11.6 ± 2.8[b]
during VL	9.0 ± 2.6	8.3 ± 3.2	
after VL	25.2 ± 3.6[d]	15.6 ± 4.6	
N of females	22	16	17

[a] for all measures mean (± SEM)
[b] $p < .05$, one-way ANOVA followed by Student-Newman-Keuls procedure
[c] $p < .05$, sign test
[d] $p < .01$, sign test

of whether the animals copulated, solicited males more and exhibited higher levels of locomotor activity than females tested with non-vocalizing males. The effects of ultrasounds on female proximity score appeared confounded with copulation, since only females tested with copulating and vocalizing males had a significantly higher proximity score than females tested with non-vocalizing males.

A clearer indication of the possible facilitation by ultrasounds of female proceptive behavior was observed by comparing female behavior before and after performance of the first ultrasound in both groups of females tested with vocalizing males. It is noteworthy that solicitation rate, locomotor activity, and proximity exhibited during the VL by both groups of females tested with vocalizing males were very similar to that exhibited by females tested with non-vocalizing males. Both groups of females experiencing male vocalizations increased their locomotor activity following production of the first ultrasound. Additionally, the proximity score increased significantly after the VL in females tested with vocalizing and copulating males, but did not reach significance in females tested with vocalizing and non-copulating males. Solicitation rate remained unchanged in both groups of females.

Figure 6 illustrates comparisons of female proceptive behavior during different temporal periods in females tested with vocalizing and copulating males. Females spent a greater percentage of time both actively moving about the cage and in close proximity to the male during the PVIL than during the period before the first ultrasound (Sign tests, $p<.001$). Since solicitation rate did not vary significantly during these time periods, the increase in solicitation

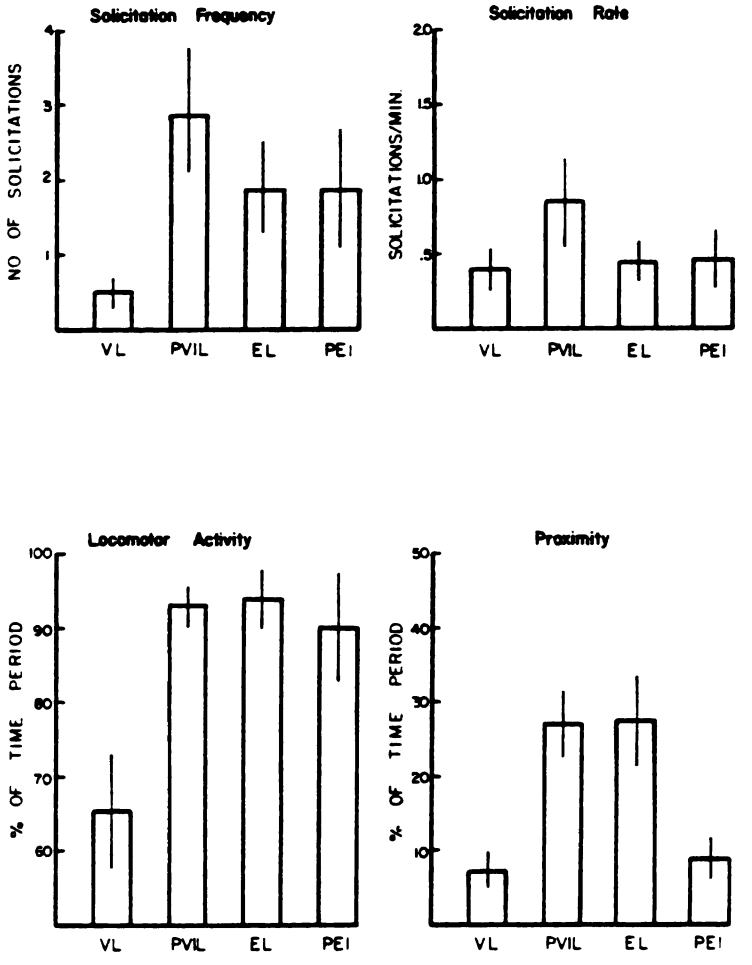

Figure 6. Mean (±SEM) solicitation frequency, solicitation rate, % locomotor activity, and % proximity observed during different temporal periods of the sexual behavior test in sexually receptive female deer mice tested with vocalizing and copulating males.
Note. VL=vocalization latency, PVIL=postvocalization intromission latency, EL=ejaculation latency, and PEI=postejaculatory interval.

frequency observed during the PVIL was due to the fact that the PVIL time span was longer than the VL. Levels of female proceptive behavior remained unchanged after the animals began copulating. Finally, during the PEI, females spent considerably less time in close proximity to the male (Sign test, $p<.001$), even though levels of locomotor activity remained high.

Discussion

After having investigated in separate experiments, sexual behaviors of male and female deer mice, a strictly behavioral description of the sequence of events taking place during sexual behavior tests may prove useful. Generally, after a receptive female was introduced into the male's cage, she would move toward the male's nest and occupy it, often displacing the male from the nest in the process. Initially, she seldom left the nest. However, with the occurrence of male ultrasonic vocalizations, the female increased her locomotor activity, circling the male often, and occasionally, soliciting the male. Concurrent with this increase in female behavior, male ultrasound production remained high. Also, male solicitations of the female became much more prevalent. During this period, the male spent a great deal of time chasing the female. Normally, she would allow the male to approach and sniff her, before darting away from him. Often the female bit or barked audibly at the male pursuing her. Nevertheless, male pursuit of the female eventually led to copulation.

During copulation, precopulatory patterns of behavior were generally maintained, especially male solicitations and female darting. However, several notable departures from this pattern were observed. Male vocalizations declined dramatically, often ceasing completely. Also, male solicitations usually resulted in successful intromissions with female lordosis. After each intromission, the male groomed himself before resuming his solicitations and copulation with the female.

Following ejaculation, the female continued to move about the cage. However, the behavior of the male changed dramatically. He became quite inactive, spending most of his time in the corner of the cage, grooming himself and vocalizing. It was not until several minutes after ejaculation that the male stopped grooming and again started to solicit the female, so that additional copulatory series could occur.

It is well established that female locomotor activity increases during estrus (Dewsbury, 1967; Richter, 1947). This increased locomotor activity is only one reflection of the many estrogen-induced changes in female behavior (Wade, 1976). Especially noteworthy in the present study was the ability of female deer mice to regulate their activity during estrus, on the basis of whether the male vocalized. Under natural conditions, when a receptive female enters into the territory of another male, it would seem adaptive for the female to seek out a safe place from which she could assess the intentions of the male toward her. Will he react to her presence in a sexual or aggressive fashion? Although not directly analogous to the natural

situation, in the laboratory, female deer mice placed in the male's cage, markedly increased their locomotor activity and became much more accessible to the male only after the male began producing ultrasounds. Conceivably, males signalled both their non-aggressiveness and also their sexual interest to the female by producing ultrasonic vocalizations. After the female perceived these sexual signals by the male, it is quite possible that her reluctance to move about the cage and exhibit proceptive behavior dissipated.

It is important to note that in many tests, copulation did not occur despite the fact that males produced ultrasonic vocalizations and females exhibited proceptive behavior. This may have been due to the fact that after prouducing their first ultrasound, males that failed to copulate did not solicit females as frequently as males that went on to copulate (Experiment 3, Part B). These vocalizing but non-copulating males appeared to perform the initial requisite sexual responses; however, they did not progress to more advanced sexual behaviors. Since males had at most only two previous sessions with receptive females, their lack of solicitations may have been a result of their sexual inexperience. Alternatively, their failure to solicit females may have been a consequence of being rebuffed by the female. Perhaps by acting aggressively toward males on their initial solicitations, females may further insure that they mate with a reproductively fit individual.

During the period from first ultrasound to first intromission, the distance maintained between the mating pair was reduced from the level observed before the first ultrasound. This high degree of proximity seen in the mating pair during the PVIL obviously

facilitated the initiation of copulation. It also appeared to be correlated with elevated male vocalization rate and male solicitation rate. In pairs of deer mice in which males vocalized but copulation did not occur, following the production of the first ultrasound, proximity scores, as well as male vocalization and male solicitation rate did not reach the levels attained by copulating pairs. Most likely, the combination of male vocalizations and male solicitations, rather than either behavior alone, contributed to the closeness maintained between the male and female. In other rodent species, proximity scores and ultrasounds have not been monitored concurrently during sexual behavior tests. Nevertheless, both female hamsters and female house mice exhibited a preference to be near an ultrasounding animal or sound source (Floody & Pfaff, 1977b; Nunez et al., in preparation).

The decline in male vocalizations observed during copulation was not accompanied by any apparent changes in female behavior. Rather, with the exception of the occurrence of lordosis, females continued to exhibit the same circling and darting behavior which they had exhibited immediately preceding copulation. Conceivably, the copulatory behavior itself was a sufficient stimulus to maintain female behavior through ejaculation. Moreover, in other rodent species, such as house mice, hamsters, and collared lemmings there are similar reports that ultrasonic vocalizations waned once the animals began copulating (Brooks and Banks, 1973; Floody et al., 1977; Sales, 1972). These observations have also fostered the notion that ultrasonic vocalizations of rodents serve primarily a courtship function (Nyby & Whitney, 1978; Sales & Pye, 1974).

After male deer mice ejaculated, there was a resumption of ultrasonic calling. This calling occurred despite an apparent lack of interest in the female by the male, as evidenced by his substantially reduced locomotor activity and solicitation rate. Previously, it was suggested that these postcopulatory vocalizations served to maintain female arousal, in order that additional copulatory series could be achieved (see Discussion, Experiment 3). The observation that female locomotor activity remained high during the postejaculatory period provides further support for this notion. However, since the amount of time the male and female spent near one another declined during the PEI, the postejaculatory vocalizations may also be signalling the female to stay away from the male while he is in a so called "down-state" (Adler & Aniko, 1979; Barfield & Geyer, 1975). These two functions for postejaculatory vocalizations are not mutually exclusive. Furthermore, although no differences in the nature (features) of the ultrasounds were noted during the different temporal periods of the sexual behavior test, it is quite possible that the ultrasound monitoring equipment may not have been able to detect subtle differences which may have existed.

Finally, in concluding this discussion, it is important to stress that the functional significance of deer mice ultrasonic communication was investigated using an indirect approach based on correlational evidence. Conclusions derived from correlational evidence are at best tentative, until additional experiments are performed to directly analyze the consequences of male ultrasounds. Such future work should examine the experimental effects of muting the male, deafening the

female, and varying physical charateristics of male ultrasonic vocalizations.

SUMMARY AND CONCLUSIONS

From the results of the present experiments, the following conclusions concerning the relationship of ultrasonic vocalizations to deer mice sexual behavior were derived:

(1) Ultrasonic vocalizations during sexual behavior tests were sexually dimorphic such that only males produced ultrasounds (Exp. 1).

(2) Males that copulated always produced ultrasonic vocalizations, whereas males failing to copulate seldom produced ultrasonic vocalizations (Exp. 1).

(3) Endocrine status of the female influenced male ultrasonic calling. Males vocalized with sexually receptive females, but rarely vocalized with ovariectomized females (Exp. 2).

(4) An anesthetized sexually receptive female was a sufficient stimulus to elicit male ultrasonic calling. Most likely, olfactory cues triggered ultrasound production in this situatiion (Exp. 2).

(5) A courtship role for precopulatory ultrasonic vocalizations was indicated by the observation that males always produced ultrasonic vocalizations before the onset of copulatory behavior (Exp. 1).

(6) As evidenced by increases in male solicitation rate, female locomotor activity, and in the proximity maintained between the male and female, precopulatory ultrasonic vocalizations were associated with both elevated levels of male sexual behavior and female proceptive behavior (Exp. 3 & Exp. 5).

(7) Male ultrasonic vocalizations did not appear to play an important role during copulation, since vocalization rate declined during this period, while other measures of male and female sexual behavior remained high (Exp. 3 & Exp. 5).

(8) Following ejaculation, male ultrasonic vocalizations may have served to sustain the interest of the female in the male during a time when the male was in a withdrawn state (Exp. 3 & Exp. 5).

(9) Gonadal hormones influenced male ultrasonic calling. Testosterone, as well as two of its major metabolites, dihydrotestosterone and estradiol, restored male ultrasonic calling in long-term castrated males (Exp. 4).

(10) Combined treatment of castrated males with subthreshold dosages of both dihydrotestosterone and estradiol activated male ultrasonic calling, as well as male copulatory behavior. This observation further supported the hypothesis that testosterone may stimulate male sexual behavior by being metabolized to both its reduced and aromatized products (Exp. 4).

REFERENCE NOTES

1. Communication involving visual cues is apparently of minimal importance in rodents. No study has yet to find any deficits in mating behavior following blinding of one of the sexual partners (Beach, 1942; Hard & Larsson, 1968; Kow & Pfaff, 1976). The possi bility that gustatory stimulation influences rodent reproductive behaviors has not been adequately investigated. Both autogrooming and allogrooming are often observed during sexual behavior testing (Dewsbury, 1967; 1978); however, the functional significance of these behaviors remains unclear.
2. Heretofore, female locomotor activity has not been considered as a distinct category of proceptive behavior. Rather components of female locomotor activity such as hopping, darting, and soliciting are commonly used as measures of proceptive behavior (Beach, 1976; Madlafousek & Hlinak, 1977). The locomotor activity of female dee mice often included a category of proceptive behavior that Beach (1976) termed "alternating approach and withdrawal". Although solicitations are one form of approach and withdrawal behavior, female deer mice often approached and withdrew from the male witho getting within 1 body length of him.
3. Proximity was judged to be a measure of female proceptive behavior because pilot observations indicated that female deer mice appear to exert a greater control over the distance maintained between th mating pair than males. Females were easily able to prevent males from getting close to them by fending off, biting, or running away from soliciting males.

LIST OF REFERENCES

LIST OF REFERENCES

Adler, N. T. The behavioral control of reproductive physiology. In W. Montagna and W. A. Sadler (Eds.), Reproductive behavior. New York: Plenum Press, 1974.

Adler, N. T. Social and environmental control of reproductive processes in animals. In T. McGill, D. Dewsbury, and B. Sachs (Eds.), Sex and behavior: Status and prospectus. New York: Plenum Press, 1978.

Adler, N. T., & Anisko, J. J. The behavior of communicating: An analysis of the 22 kHz call of rats (Rattus norvegicus). Amer. Zool., 1979, 19, 493-508.

Adler, N. T., & Zoloth, S. R. Copulatory behavior can inhibit pregnancy in female rats. Science, 1970, 168, 1480-1482.

Allin, T. J., & Banks, E. M. Effects of temperature on ultrasound production by infant albino rats. Develop. Psychobiol., 1971, 4, 149-156.

Allin, T. J., & Banks, E. M. Functional aspects of ultrasound production by infant albino rats. Anim. Behav., 1972, 20, 175-185.

Anisko, J. J., Adler, N. T., & Suer, S. F. Patterns of postejaculatory urination and sociosexual behavior in the rat. Behav. Neur. Biol., 1979, 26, 169-176.

Anisko, J. J., Suer, S. F., McClintock, M. K., & Adler, N. T. Relation between 22 kHz ultrasonic signals and sociosexual behavior in rats. J. Comp. Physiol. Psychol., 1978, 92, 821-829.

Barfield, R. J., Auerbach, P., Geyer, L. A., & McIntosh, T. K. Ultrasonic vocalizations and rat sexual behavior. Amer. Zool., 1979, 19, 469-480.

Barfield, R. J., & Geyer, L. A. Sexual behavior: Ultrasonic postejaculatory song of the male rat. Science, 1972, 176, 1349-1350.

Barfield, R. J., & Geyer, L. A. The ultrasonic postejaculatory vocalization and the postejaculatory refractory period of the male rat. J. Comp. Physiol. Psychol., 1975, 88, 723-734.

Barfield, R. J., & Krieger, M. S. Ejaculatory and postejaculatory behavior of male and female rats: Effects of sex hormones and electric shock. Physiol. Behav., 1977, 19, 203-208.

Beach, F. A. Characteristics of masculine "sex drive". In M. R. Jones (Ed.), The Nebraska symposium of motivation. Lincoln: University of Nebraska Press, 1956.

Beach, F. A. Sexual attractivity, proceptivity, and receptivity in female mammals. Horm. Behav., 1976, 7, 105-138.

Beach, F. A., Stern, B., Carmichael, M., & Ranson, E. Comparisons of sexual receptivity and proceptivity in female hamsters. Behav. Biol., 1976, 18, 473-487.

Bean, N. J. Olfactory and vomeronasal mediation of ultrasonic vocalizations in male mice. Physiol. Behav., 1982, in press.

Bean, N. J., Nunez, A. A., & Conner, R. Effects of medial preoptic lesions on male mouse ultrasonic vocalizations and copulatory behavior. Brain Res. Bull., 1981, 6, 109-112.

Bell, R. W. Ultrasounds in small rodents: Arousal produced and arousal producing. Develop. Psychobiol., 1974, 7, 39-42.

Bell, R. W. Ultrasonic control of maternal behavior: Developmental implications. Amer. Zool., 1979, 19, 413-418.

Bell, R. W., Nitschke, W., Gorry, T. H., & Zachman, T. A. Infantile stimulation and ultrasonic signalling: A possible mediator of early handling phenomena. Develop. Psychobiol., 1971, 4, 181-192.

Bell, R. W., Nitschke, W., & Zachman, T. A. Ultrasounds in three three strains of mice. Behav. Biol., 1973, 8, 805-814.

Bronson, F. H. Rodent pheromones. Biol. Reprod., 1971, 4, 344-357.

Bronson, F. H., & Caroom, D. Preputial gland of the male mouse: Attractant function. J. Reprod. Fert., 1971, 25, 279-282.

Brooks, R. J., & Banks, E. M. Behavioral biology of the collared lemming [Dicrostonyx groelandicus (Traill)]: An analysis of acoustic communication. Anim. Behav. Monog., 1973, 6, 1-82.

Brown, A. M., & Pye, J. D. Auditory sensitivities at high frequencies in mammals. Adv. Comp. Physiol. Biochem., 1975, 6, 1-73.

Brown, R. E. The 22-kHz pre-ejaculatory vocalizations of the male rat. Physiol. Behav., 1979, 22, 483-489.

Bruchovsky, N., & Wilson, J. D. The conversion of testosterone to 5α-androstan-17β-ol-3-one by rat prostate in vivo and in vitro. J. Biol. Chem., 1968, 243, 2012-2021.

Callard, G. V., Petro, Z., & Ryan, K. J. Conversion of androgen to estrogen and other steroids in the vertebrate brain. Amer. Zool., 18, 511-523.

Caroom, D., & Bronson, F. H. Responsiveness of female mice to preputial attractant: Effects of sexual experience and ovarian hormones. Physiol. Behav., 1971, 7, 659-662.

Clemens, L. G., & Pomerantz, S. M. Male sexual behavior in deer mice (Peromyscus maniculatus bairdi) following castration and hormone replacement. Horm. Behav., 1981, 15, 183-196.

Clemens, L. G., & Pomerantz, S. M. Testosterone acts as a prohormone in male deer mice (Peromyscus maniculatus bairdi) to stimulate male copulatory behavior. J. Comp. Physiol. Psychol., 1982, in press.

Colvin, M. A. Analysis of acoustic structure and function in ultrasounds of neonatal Microtus. Behavior, 1973, 44, 234-263.

Conrad, L. C. A., & Pfaff, D. W. Autoradiographic study of efferents from medial basal forebrain and hypothalamus in the rat. I. Medial preoptic area. J. Comp. Neurol., 1976a, 169, 185-220.

Conrad, L. C. A., & Pfaff, D. W. Autoradiographic study of efferents from medial basal forebrain and hypothalamus in the rat. II. Medial anterior hypothalamus. J. Comp. Neurol., 1976b, 169, 221-162.

DeBold, J. F., & Clemens, L. G. Aromatization and the induction of male sexual behavior in male, female, and androgenized female hamsters. Horm. Behav., 1978, 11, 401-413.

DeFries, J. C., & McClearn, G. E. Social dominance and Darwinian fitness in the laboratory mouse. Amer. Natural., 1970, 104, 408-411.

Dewsbury, D. A. A quantitative description of rats during copulation. Behavior, 1967, 29, 154-177.

Dewsbury, D. A. Copulatory behavior of deer mice (Peromyscus maniculatus bairdi): III. Effects on pregnancy initiation. J. Comp. Physiol. Psychol., 1979, 93, 178-188.

Dizinno, G., & Whitney, G. Androgen influence on male mouse ultrasounds during courtship. Horm. Behav., 1976, 7, 188-192.

Dizinno, G., Whitney, G., & Nyby, J. Ultrasonic vocalizations by male mice (Mus musculus) in response to female sex pheromone: Experiential determinants. Behav. Biol., 1978, 22, 104-113.

Doty, R. D. Odor preferences of female Peromyscus maniculatus bairdi for male mouse odors of P. m. bairdi and P. leucopus noveboracensis as a function of estrous state. J. Comp. Physiol. Psychol., 1972, 81, 191-197.

Floody, O. R. The hormonal control of ultrasonic communication in rodents. Amer. Zool., 1981, 21, 129-142.

Floody, O. R., Merkle, D. A., Cahill, T. J., & Shopp, Jr., T. J. Gonadal hormones stimulate ultrasound production by female hamsters. Horm. Behav., 1979a, 12, 172-184.

Floody, O. R., & O' Donohue, T. L. Lesions of the mesencephalic central gray depress ultrasound production and lordosis by female hamsters. Physiol. Behav., 1980, 24, 79-85.

Floody, O. R., & Pfaff, D. W. Communication among hamsters by high-frequency acoustic signals. I. Physical characteristics of hamster calls. J. Comp. Physiol. Psychol., 1977a, 91, 794-806.

Floody, O. R., & Pfaff, D. W. Communication among hamsters by high-frequency acoustic signals. II. Determinants of calling by males and females. J. Comp. Physiol. Psychol., 1977b, 91, 820-829.

Floody, O. R., Pfaff, D. W., & Lewis, C. D. Communication among hamsters by high-frequency acoustic signals. III. Responses evoked by natural and synthetic calls. J. Comp. Physiol. Psychol., 1977, 91, 807-819.

Floody, O. R., Walsh, C., & Flanagan, M. T. Testosterone stimulates ultrasound production by male hamsters. Horm. Behav., 1979b, 12, 164-171.

Geyer, L. A., & Barfield, R. J. Influence of gonadal hormones and sexual behavior on ultrasonic vocalizations in rats: I. Treatment of females. J. Comp. Physiol. Psychol., 1978, 92, 438-446.

Geyer, L. A., Barfield, R. J., & McIntosh, T. K. Influences of gonadal hormones and sexual behavior on ultrasonic vocalizations in rats: II. Treatment of males. J. Comp. Physiol. Psychol., 1978a, 92, 447-456.

Geyer, L. A., McIntosh, T. K., & Barfield, R. J. Effects of ultrasonic vocalizations and male's urine on female rat readiness to mate. J. Comp. Physiol. Psychol., 1978b, 92, 457-462.

Green, S., & Marler, P. The analysis of animal communication. In P. Marler & J. G. Vandenburgh (Eds.), Handbook of behavioral neurobiology (Vol. 3), Social behavior and communication. New York: Plenum Press, 1979.

Holman, S. D. Sexually dimorphic, ultrasonic vocalizations of Mongolian gerbils. Behav. Neur. Biol., 1980, 28, 183-192.

Holman, S. D. Neonatal androgenic influences on masculine ultrasonic vocalizations of Mongolian gerbils. Physiol Behav., 1981, 26, 583-586.

Johnston, R. E. Sexual attraction function of golden hamster vaginal secretion. Behav. Biol., 1974, 12, 111-117.

Johnston, R. E. Sexual excitation function of hamster vaginal secretion. Anim. Learn. Behav., 1975, 3, 161-166.

Johnston, R. E. The causation of two scent marking behavior patterns in female hamsters (Mesocricetus auratus). Anim. Behav., 1977, 17, 317-327.

Johnston, R. E. Olfactory preferences, scent marking, and "proceptivity" in female hamsters. Horm. Behav., 1979, 13, 21-39.

Jurgens, U., & Pratt, S. R. Role of the periaqueductal grey in vocal expressions of emotion. Brain Res., 1979, 167, 367-378.

Kairys, D. J., Magalhaes, H., & Floody, O. R. Olfactory bulbectomy depresses ultrasound production and scent marking by female hamsters. Physiol. Behav., 1980, 25, 143-146.

Kelley, D. B. Social signals - an overview. Amer. Zool., 1981, 21, 111-116.

Kurtz, R., & Adler, N. T. Electrophysiological correlates of sexual behavior in the male rat. J. Comp. Physiol. Psychol., 1973, 84, 225-239.

Lieberburg, I., & McEwen, B. S. Estradiol 17β: A metabolite of testosterone recovered in brain cell nuclei from limbic areas of adult male rat brains. Brain Res., 1975, 91, 171-174.

Lore, R. K., Flannelly, K., & Farina, P. Ultrasounds produced by rats accompany decreases in intraspecific fighting. Aggressive Behav., 1976, 2, 175-181.

Luttge, W. G. Endocrine control of mammalian male sexual behavior: an analysis of the potential role of testosterone metabolites. In C. Beyer (Ed.), Endocrine control of sexual behavior. New York: Raven Press, 1979.

Madlafousek, J., & Hlinak, Z. Sexual behavior of the female laboratory rat: Inventory, patterning, and measuremnet. Behavior, 1977, 63, 129-174.

Maier, S. F., & Seligman, M. E. P. Learned helplessness: Theory and evidence. J. Exp. Psychol.: General, 1976, 105, 3-46.

Marler, P. The evolution of communication. In T. A. Sebeok (Ed.), How animals communicate. Bloomington: Indiana University Press, 1976.

Massa, R., Justo, S., & Martini, L. Conversion of testosterone into 5α-reduced metabolites in the anterior pituitary and the brain of maturing rats. J. Steroid Biochem., 1975, 6, 567-572.

McIntosh, T. K., & Barfield, R. J. The temporal patterning of 40-60 kHz ultrasonic vocalizations and copulation in the rat (Rattus norvegicus). Behav. Neur. Biol., 1980, 29, 349-358.

McIntosh, T. K., Barfield, T. K., & Geyer, L. A. Ultrasonic vocalizations facilitate sexual behavior of female rats. Nature, 1978, 272, 163-164.

Murphy, M. R. Effects of female hamster vaginal discharge on the behavior of male hamsters. Behav. Biol., 1973, 9, 367-375.

Murphy, M. R. Olfactory impairment, olfactory bulb removal, and mammalian reproduction. In R. L. Doty (Ed.), Mammalian olfaction, reproductive processes, and behavior. New York: Academic Press, 1976.

Noirot, E. Ultrasounds in young rodents I. Changes with age in albino mice. Anim. Behav., 1966, 14, 459-462.

Noirot, E. Ultrasounds and maternal behavior in small rodents. Develop. Psychobiol., 1972, 5, 371-387.

Noirot, E., Goyens, J., & Buhot, M. Aggressive behavior of pregnant mice towards males. Horm. Behav., 1975, 6, 9-17.

Nunez, A. A., Bean, N. J., & Pomerantz, S. M. Female mice exhibit a preference for ultrasounding males. In preparation.

Nunez, A. A., Nyby, J., & Whitney, G. The effects of testosterone, estradiol, and dihydrotestosterone on male mouse (Mus musculus) ultrasonic vocalizations. Horm. Behav., 1978, 11, 264-272.

Nyby, J., Dizinno, G., & Whitney, G. Social status and ultrasonic vocalizations of male mice. Behav. Biol., 1976, 18, 285-289.

Nyby, J., Dizinno, G., & Whitney, G. Sexual dimorphism in ultrasonic vocalizations of mice (Mus musculus): Gonadal hormone regulation. J. Comp. Physiol. Psychol., 1977a, 91, 1424-1431.

Nyby, J., & Whitney, G. Ultrasonic communication of adult myomorph rodents. Neurosci. Biobehav. Rev., 1978, 2, 1-14.

Nyby, J., Whitney, G., Schmitz, G., & Dizinno, G. Postpubertal experience establishes signal value of mammalian sex odor. Behav. Biol., 1978, 22, 545-552.

Nyby, J., Wysocki, C. J., Whitney, G., & Dizinno, G. Pheromonal regulation of male mouse ultrasonic courtship (Mus musculus). Anim. Behav., 1977b, 25, 333-341.

Nyby, J., Wysocki, C. J., Whitney, G., Dizinno, G., & Schneider, J. Elicitation of male mouse (Mus musculus) ultrasonic vocalizations: I. Urinary cues. J. Comp. Physiol. Psychol., 1979, 93, 957-975.

Nyby, J., Wysocki, C. J., Whitney, G., Dizinno, G., Schneider, J., & Nunez, A. A. Stimuli for male mouse (Mus musculus) ultrasonic courtship vocalizations: Presence of female chemosignals and/or absence of male chemosignals. J. Comp. Physiol. Psychol., 1981, 95, 623-629.

Parrott, R. F. An effect of testosterone on sexual arousal in the rat, determined from records of ultrasonic vocalization before and after castration. Physiol. Behav., 1976, 16, 689-692.

Parrott, R. F., & Barfield, R. J. Post-ejaculatory vocalization in castrated rats treated with various steroids. Physiol. Behav., 1975, 15, 159-163.

Pfaff, D. W. Estrogens and brain function. New York: Springer-Verlag, 1980.

Pfaff, D. W., & Keiner, M. Atlas of estradiol-concentrating cells in the central nervous system of the female rat. J. Comp. Neurol., 1973, 151, 121-158.

Pollak, E. I., & Sachs, B. D. Excitatory and inhibitory effects of stimulation applied during the post-ejaculatory interval of the male rat. Behav. Biol., 1975, 15, 449-462.

Pomerantz, S. M., Nunez, A. A., & Bean, N. J. Inferior laryngeal nerves arising fom the nucleus ambiguus control ultrasonic vocalizations in male house mice (Mus musculus). In preparation.

Richter, C. P. Hormones and rhythms in man and animals. Rec. Prog. Horm. Res., 1947, 8, 105-159.

Roberts, L. H. Evidence for the laryngeal source of ultrasonic and audible cries of rodents. J. Zool., 1975, 175, 243-257.

Sachs, B. D., & Barfield, R. J. Functional analysis of masculine copulatory behavior in the rat. In J. Rosenblatt, R. Hinde, E. Shaw, & C. Beer (Eds.), Advances in the study of behavior, Vol. III. New York: Academic Press, 1976.

Sakuma, Y., & Pfaff, D. W. Facilitation of female reproductive behavior from mesencephalic central grey in the rat. Amer. J. Physiol., 1979a, 237, R278-284.

Sakuma, Y., & Pfaff, D. W. Mesencephalic mechanisms for integration of female reproductive behavior in the rat. Amer. J. Physiol., 1979b, 237, R285-290.

Sales, G. D. Ultrasound and mating behavior in rodents with some observations of other behavioral situations. J. Zool., 1972, 168, 149-164.

Sales, G. D. Ultrasound and aggressive behavior in rats and other small mammals. Anim. Behav., 1972b, 20, 88-100.

Sales, G. D. Strain differences in the ultrasonic behavior of rats (Rattus norvegicus). Amer. Zool., 1979, 19, 513-527.

Sales, G. D., & Pye, D. Ultrasonic communication by animals. London: Chapman & Hall, 1974.

Sales, G. D., & Smith, J. C. Comparative studies of ultrasonic calls of infant murid rodents. Develop. Psychobiol., 1978, 11, 595-619.

Sar, M., & Stumpf, W. E. Distribution of androgen target cells in rat forebrain and pituitary after 3[H]-dihydrotestosterone administration. J. Steroid Biochem., 1977, 8, 1131-1135.

Smith, W. J. The behavior of communicating: An ethological approach. Cambridge: Harvard University Press, 1977.

Smotherman, W. P., Bell, R. E., Starzac, J., Elias, J., & Zachman, T. A. Maternal responsiveness to infant vocalization and olfactory cries in rats and mice. Behav. Biol., 1974, 12, 55-66.

Thiessen, D. D., Friend, H. C., & Lindzey, G. Androgen control of territorial marking in the Mongolian gerbil. Science, 1968, 160, 432-434.

Thiessen, D. D., Kittrell, E. M. W., & Graham, J. M. Biomechanics of ultrasound emission in the Mongolian gerbil, Meriones unguiculatus. Behav. Neur. Biol., 1980, 29, 415-429.

Thomas, D. A., Howard, S. B., & Barfield, R. J. The influence of 50 kHz mating calls produced by male rats on mating patterns exhibited by females. Paper presented at Conference on Reproductive Behavior, Nashville, TN., 1981.

Wade, G. N. Sex hormones, regulatory behavior, and body weight. In J. S. Rosenblatt, R. A. Hinde, E. Shaw, & C. G. Beer (Eds.), Advances in the study of behavior, Vol. 6. New York: Academic Press, 1976.

Wetzel, D. M., Kelley, D. B., & Campbell, B. A. Central control of ultrasonic vocalizations in neonatal rats: I. Brain stem motor nuclei. J. Comp. Physiol. Psychol., 1980, 94, 596-605.

Whalen, R. E., & DeBold, J. F. Comparative effectiveness of testosterone, androstenedione and dihydrotestosterone in maintaining mating behavior in the castrated male hamster. Endocrinology, 1974, 95, 1674-1679.

Whalen, R. E., & Rezek, D. L. Localization of androgenic metabolites in the brain of rats administered testosterone or dihydrotestosterone. Steroids, 1972, 20, 717-725.

Whitney, G., Alpern, M., Dizinno, G., & Horowitz, G. Female odors evoke ultrasounds from male mice. Anim. Learn. Behav., 1974, 2, 13-18.

Whitney, G., Coble, J. R., Stockton, M. D., & Tilson, E. F. Ultrasonic emissions: Do they facilitate courtship in mice? J. Comp. Physiol. Psychol., 1973, 84, 445-452.

Yajima, Y., Hayashi, Y., & Yoshi, N. The midbrain central gray substance as ahighly sensitive neural structure for the production of ultrasonic vocalization in the rat. Brain Res., 1980, 198, 446-452.