THE EFFECTS OF ULTRASOUND ON THE BEHAVIOUR OF COMMENSAL RODENTS WITH A DISCUSSION OF ITS POTENTIAL IN MANAGEMENT AND CONTROL PROGRAMS

bу

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ABSTRACT

Responses of commensal rodents to natural and man-made ultrasound were considered in this study. Both live and recorded ultrasonic vocalizations of an isolated mouse pup elicited retrieval behaviour from lactating females. Rapid waning of responses towards sound alone indicates that other stimuli may also be involved. Juvenile <u>Fattus norvegicus</u> emitted long (400 - 800 ms) ultrasonic cries (26 kHz) when exposed to threatening stimuli but not when exposed to painful stimuli. Ultrasonic signals were not detected from similarly treated adult R. norvegicus, R. rattus or <u>Mus musculus</u>.

In laboratory studies, rats and mice were initially repelled by intense ultrasound (20 kHz, 130 db at 1 m). Rats became habituated to the sound over a period of days. The repelling effect waned more rapidly with mice, but deafening as well as habituation was involved.

Rats feeding in a small warehouse were completely repelled for a period of days by high intensity ultrasound, but were not driven away. Rats burrowing and feeding in a chicken barn showed reduced activity but were not driven off when exposed to similar levels of ultrasound. Pomestic rooster chicks maintained growth rates in intense ultrasonic fields, but a domestic pig showed ill-effects. The role of ultrasound in natural behaviour and its application in rodent management is discussed.

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TABLE OF CONTENTS

																							Page
Examinin	g Commi	ttee	A	.pp	ro	va	11		•	•									•			•	ii
Abstract									•	•													iii
Acknowle	dgement	s .																	•				iv
Table of	Conten	ts.																	•			•	v
List of	Tables																						viii
List of	Figures									•								•	•				ix
General	Introdu	ctio	n						•														1
General 1	Methods	and	M	at	er	ia	ls			•		•		•									6
Chapter		Eff Comm																					9
Int	roducti	on.			•					•	•		•				•					•	9
1.	Ultras Lactat																	•				•	14
	Object	ive			•	•				•		•					•			•		•	14
	Method	s a n	d :	Ma.	t e	ri	al	s		•	•	•	•			•	•		•	•	•	•	14
	Result	s.								•	•	•	•	•			•	•	•			•	18
2.	Stress mensal														ti •	.or	ıs •	fı	on.	ı (on •	n- •	21
	Object	ive	•			•	•			•						•	•	•				•	21
	Method	s a n	d l	Ma	te:	ri	al	s	•									•	•				21
	Result	s.	•									••						•	•	•	•		24
Disc	cussion					•								•		•					•	•	58
3.	Immedia Rats.				ct:	s •	of •		ig •	;h	Ir.	te •	ens •	it.	.	U1 •	tı •	ra s	ov •	nd.		n •	32
	Object	ive	•	•	•	•			•	•	•	•		•			•	•		•		•	32
	Method	s an	d l	Ma 1	te	ri	al	s	•	•		•		•			•	•		•	•	•	32
	Pagult	c														•							34

																					rage
	4.	Effec Behav	ts o	f Hi	igh Rat	In ts:	ter F	nsi Par	ty t :	υ: 1.	ltr •	as •	ou •	nd •	•	n •	tr •	e •			35
		Objec	tive																		35
		Metho	ds ar	nd N	Mat:	eri	als	s .	•												35
	5.	Effec Behav	ts o: iour	f H	igh R a t	In	ter F	nsi Par	ty t 2	υ] 2.	ltr •	as •	ou •	nd •	•	n •	tr •	ne •			39
		Objec	tive			•															39
		Metho	ds aı	nd N	1ate	eria	als	s .						•			•			•	39
	Res	ults .													•						40
	6.	Effec Beh a v	ts of	f Hi	igh R a t	In	ter F	si ar	ty t 3	υ1 3.	ltr •	as •	ou •	nd	•	n •	th •	e •		•	47
		Objec	tive													•				•	47
		Metho	ds ar	nd N	la te	eria	als														47
		Resul	ts.																		48
	Disc	cussio	n					•		•			•	•		•		•	•	•	51
	7.	Repel																			•
		on <u>Mu</u>	s mus	scul	us	•	• •	•	•	•	•	•	•	•	•	•	•	•	•	•	55
		Objec	tive		•	•		•	•	•	•	•	•	•	•	•	•	•	•	•	55
		Metho	ds ar	nd M	late	erie	als	•	•	•	•	•	•	•	•	•	•	•	•	•	55
		Resul	ts.		•			•	•	•	•	•	•	•	•	•	•	•	•	•	61
		Discu	ssior	n				•	•	•	•	•	•	•		•	•	•	•	•	69
Chapt	er]	II. F	ield	Tes	ts	of	an	U	Ltr	as	on	ic	W:	hi	st	le	•	•	•	•	74
	Inti	roduct	ion.		•			•	•	•		•	•	•	•	•	•	•	•	•	74
	1.	Repel	lent	Eff	ect	s	of	Hi	gh	In	te	ns	it	У	Ul	tr	as	ou	nd		5 (
		on Ra	ts ir	a	War	eho	ous	e.	•	•	•	•	•	•	•	•	•	•	•	•	76
		Objec	tive		•			•	•	•	•	•	•	•	•	•	•	•	•	•	76
		Test	Site	and	Me	the	ods					•	•	•	•	•	•	•	•	•	76

																									ŀ	age
2.		Rep																								78
	(Obj	ect	iv	е				•	•	•		•		•	•	•	•	•	•	•		•		•	78
	ı	Tes	t S	Sit	e ·	ar	ıd	Μe	etł	nod	ls	•	•	•	•		•	•	•		•				•	78
3.		Eff Gro																							•	81
		Obj	ect	iv	e	•	•		•	•	•	•	•	•	•	•	•	•	•		•	•		•		81
	1	Met	hod	ls	an	d	Ма	ıt€	eri	la]	Ls	•			•	•	•	•	•	•	•	•	•	•	•	81
4.	-	Eff Gro					_	-					-										•	•		82
		Obj	ect	iv	е			•		•		•	•	•	•	•	•		•			•	•	•	•	82
		Met	hod	ls	an	d	Ма	te	eri	ia]	Ls	•	•	•	•			•	•		•	•	•	•	•	82
R	esu	lts	•	•	•	•		•	•	•	•		•	•			•	•	•		•		•	•	•	83
D	isc	uss	ior	n.		•	•	•	•	•		•		•	•	•	•	•	•	•	•	•	•	•	•	90
Genera	1 C	onc	lus	sic	ns		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	93
Append:	ice	s.	•	•	•		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	95
Litera	tur	e C	1t	ed			•	•	•		•			•	•	•	•	•	•	•	•	•	•	•	•	102
Curric	,,7,,	m V	14 ተ 2	0 0															•	•		•				108

LIST OF TABLES

		Page
Table 1.	Sorties by lactating mice in response to live pups and recorded ultrasonic distress calls	19
Table 2.	A test of the hypothesis that there is no significant difference between the responses of lactating female mice to isolated pups and recorded distress cries of isolated pups	20
Table 3.	Juvenile male rat ultrasonic vocalizations	27
Table 4.	Rat activity in the experimental cage in Experiments 4 and 5	42
Table 5.	Rat activity in the experimental cage in Experiment 6	50
Table 6.	Repellent effects of high intensity ultrasound on mouse activity	63
Table 7.	Effects of ultrasound on the number of active burrows in the chicken barn	86

LIST OF FIGURES

			Page
Figure	1.	Oscillogram of the sound produced by the ultrasonic whistle, a spectral analysis of the same sound, and a photograph of the ultrasonic whistle and compressor	8
Figure	2.	Sonagrams of ultrasonic distress vocalizations from a 4-day-old albino mouse pup	16
Figure	3.	Apparatus for detecting, recording and analyzing ultrasonic vocalizations from rats and mice	23
Figure	4.	Sonograms of ultrasonic vocalizations produced by juvenile R. norvegicus while experimentally stressed.	25
Figure	5.	Experimental apparatus for measuring repellent effects of high intensity ultrasound on rats	37
Figure	6.	The amount of time measured in seconds per hour and seconds per visit that rats spent in the ultrasonic field	41
Figure	7.	Total amount of time that rats spent in the experimental cage and the duration of visits made by rats to the experimental cage	44
Figure	8.	Frequency of visits made by rats to the experimental cage in Experiments 4 and 5	45
Figure	9.	The total amount of time that rats spent in the experimental cage, the amount of time measured in seconds per hour and the amount of time in seconds per visit that rats spent in the ultrasound field	49
Figure	10.	Diagram of the experimental apparatus used to measure the repellent effects of high intensity ultrasound on mice	56
Figure	11.	Movements into and out of the experimental area by fed mice familiar with the experimental cage	62

			Page
Figure	12.	Movements into and out of the experimental area by mice familiar with the experimental area but deprived of food and water for 24 hours prior to the experiment	64
Figure	13.	Movements into and out of the experimental area by mice unfamiliar with the experimental area	66
Figure	14.	Macrophotographs of tympani of M. musculus damaged through exposure to high intensity ultrasound	68
Figure	15.	Floor plan of the basement of the Highway Market	7 8
Figure	16.	Floor plan of the hen house and a cross section of the pet	80
Figure	17.	Effects of ultrasound on activity of the rats in a warehouse	84
Figure	18.	Growth rate of rooster chicks in the ultrasound field	87
Figure	19.	Growth of a domestic piglet in an ultrasound field	88

GENERAL INTRODUCTION

The common brown rat, <u>Rattus norvegicus</u> Berkenhout has a long history of association with man which probably goes back to the beginning of agriculture (Barrett-Hamilton and Hinton 1920). The house mouse, <u>Mus musculus</u> L., has become commensal with man more recently (Crowcroft 1966). Competition with man has possibly led, particularly in <u>Rattus</u>, to genetic changes that have influenced behaviour (Barnett 1963). The avoidance of unfamilar objects shown by <u>R. norvegicus</u> is one example. The result of this highly adaptive behaviour is that trapping and poisoning campaigns are considered successes with 50 - 75% reductions in infestation (Davis 1953; Lindsay <u>et al.</u> 1971).

Recently, genetic resistance to the highly effective anticoagulent poisons has been noted in various locations of North America and Europe (Suttie 1973). This, in conjunction with ever reducing tolerances for losses of food and fibre to rodents, has placed new pressures on the development of more effective control measures.

Many opportunistic animals, including rodents, have highly developed diffuse motor zones in the central nervous system which give a great flexibility in response to stimuli. The all or none responses to stimuli, characteristic of lower vertebrates and invertebrates are not the only choices (Busnel 1963). The young of rodents respond to a wide variety of unspecific stimuli by escaping, but over time many of these stimuli

cease to be effective, evoking only an alerting reaction or even no response at all (Thorpe 1963). The effectiveness of other stimuli persist, with responses sharpening, particularly to stimuli that have been associated with more alarming consequences such as pain, pursuit by a predator or other noxious stimuli. In rats, the capacity of some stimuli to elicit responses corresponding to fear is reduced with increasing familiarity over a period of time. They do not, however, become habituated to other stimuli, for example being placed on a small high platform with no walls. In some cases, a stimulus loses this capacity to elicit fear very slowly as with fear of snakes in monkeys. Fear is understood to be a state, usually aroused by noxious stimulation, in which there is autonomic, especially sympathetic, activation. External signs in the rat include more-than-usual defaecation, urination, grooming and other activity. Fear, as opposed to anxiety, lasts only a short time (Barnett 1955, 1963 p. 249).

Several varieties of albino rats and mice are highly susceptible to epileptiform seizures resulting from moderately intense acoustic stimuli. These "audiogenic seizures" often result in death (Finger 1947; Frings and Frings 1952; Maier 1949; Bevan 1955; Busnel 1963). The inheritance of susceptibility is complex, probably being multigenic and quantitative (Frings et al. 1956; Henry 1973). Wild varieties of rats and mice do not show such responses to the same level of acoustic stimulus (Sprock et al. 1967).

Frings (1948), on the basis of these seizures, was the first to suggest the potential of ultrasound, or sound above 20 kilohertz (kHz) as a repellent to wild rodents. Allen et al., (1948) showed that white mice died after 1 minute exposure to a sound field of 20 kHz at 160 - 165 decibels (db) (relative to 10^{-16} watts cm²). Death results from heating or absorbing the sound. Aside from the cost involved of producing ultrasound of such enormous energy, and the danger to non-target organisms, ultrasound propagates poorly through air and is absorbed rather than reflected from any but the smoothest surfaces. Pest control by sonic destruction would not appear to have many possible applications.

Until recently, adequate audiograms showing the ability of rats and mice to hear ultrasounds were lacking. Gourevitch and Hack (1966) suggested that Wistar (an albino variety) rats had most acute hearing near 40 kHz, and Ralls (1967) reported feral Mus musculus to have most acute hearing around 20 kHz. However, Brown (1973 a, b), studying responses at the inferior collicular level, showed each species to have two peaks, one at 20 kHz and a greater one at 50 kHz for Mus; one at 8 kHz and a slightly greater one at 40 - 42 kHz for Rattus. The inferior colliculus is a rudimentary correlating centre composed of 3rd and higher order fibres, just anterior to the cerebellum. Rats are capable of echo-locating using incidental noises from movement as cues (Riley and Rosenzweig 1957). Vocalizations are not thought to be involved in this phenomenon.

Anderson (1954) was the first to report the production of pure ultrasound by small mammals other than bats. He recorded vocalizations of 25 - 28 kHz produced by caged albino rats. Zippelius and Schleidt (1956) reported ultrasonic vocalizations from the young of several species of myomorph rodents. Noirot's studies (1956, 1966, 1968, 1969) on maternal behaviour in mice and rats extended Zippelius and Schleidt's observations. Rat pups were also found to vocalize ultrasonically (Sewell 1967) and other vocalizations from adult rats were reported (Sales 1972 a, b). However, little information is available on how or even whether rodents use ultrasound in communication or on the effects of interfering with hearing.

This work reports on studies conducted on the functional role of mouse pup vocalizations. The communicative value of these vocalizations is established. Also, the vocalizations produced by isolated adult and juvenile rats and mice in stressful situations were examined for ultrasonic components. The possible communicative value of ultrasound produced in such situations is suggested. The potential of these and mouse pup vocalizations in rodent management are discussed. The repellent qualities of high intensity pneumatically produced ultrasound to rats and mice in several laboratory situations were evaluated.

In Chapter 2, the results and conclusions from two field tests of the repellent qualities of high intensity ultrasound from a commercial rodent repellent were evaluated. Also, the effects of intense ultrasound on the growth and general behaviour

of domestic chicks and a domestic pig are reported.

GENERAL METHODS AND MATERIALS

Except where otherwise indicated, all rats used in these studies were from a colony originating from three wild adult Rattus norvegicus Berkenhout trapped at a railway freight-car cleaning yard in Port Coquitlam, British Columbia. The colony was maintained in a semi-natural, earth floored enclosure 2.73 m x 2.73 m x 0.91 m. In the second year of the colony's life, six adult rats were transferred to another enclosure 2.5 m x 1.0 m x 0.91 m. A standard diet of Purina Rat Chow pellets and water was always freely available to both colonies. To minimize handling, Havahart Live Traps were used to transfer animals. Animals were never used in more than one experiment, although they were often returned to the breeding population.

Outbred albino laboratory mice <u>Mus</u> <u>musculus</u> L. and wild housemice <u>Mus</u> <u>musculus</u> L. trapped in a feed storage building near Langley, British Columbia, were maintained and bred in 46 cm x 46 cm x 15 cm sheet metal cages with 15 cm x 15 cm x 10 cm metal nest boxes. Bedding was a polyester fibre and floor litter was vermiculite. A standard diet of Purina Mouse Chow pellets and water was always freely available.

Audible and ultrasonic vocalizations of both rats and mice were detected with a Brüel and Kjaer condenser microphone, type 2360. This microphone had flat response from 8 Hz to 70 kHz ± 2 decibels (db). The signal from the microphone was amplified by a Hewlett Packard preamplifier, type 400F, at a gain that produced an output of approximately 1 Volt RMS. This signal was

recorded on a Precision Instrument tape recorder, type PI6200, at 37.5 inches per second (i.p.s.). All recordings were made on 1/4" Trubuilt magnetic recording tape, type T 18M. Ultrasonic vocalizations were visually detected at the time of recording with the VU meter of the tape recorder. Sound spectrograms of selected recorded vocalizations were made with a Kay Electric Co. Sound Spectrograph, type Sona-Graph 6061-B. In producing sonagrams, the tape playback speed was reduced from 37.5 i.p.s. to 3.75 i.p.s., effectively reducing frequencies of recordings by the same factor. This was necessary to put the ultrasonic signals within the range of the Sona-Graph. Sound levels in all studies were measured with a Brüel and Kjaer Precision Sound Level Meter, type 2203 on the linear (unweighted) network. Recorded or electronically produced ultrasonic signals for playback to animals were amplified by a Krohn-Hite IOR IOW amplifier and transduced by a Listening Corporation 10 cm condenser type loudspeaker. In one series of experiments, a variable capacitance discharge device supplied electric shocks at 100 V at several mA to the feet of the subjects.

A compressor driven ultrasonic whistle from Ultrason International, New York, was employed to produce ultrasound varying continuously between 18.5 kHz and 20.5 kHz at 130 db Sound Pressure Level (SPL) at 1 m (Fig. 1).

Figure 1

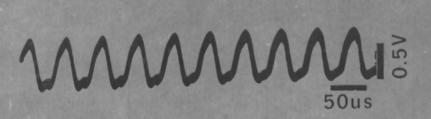
ABOVE: Oscillogram of the sound produced by one of the Ultrason whistles used in Chapter I, Sections 3 - 6, recorded with the Brüel and Kjaer 1/4" (0.635 cm) microphone at 1 m.

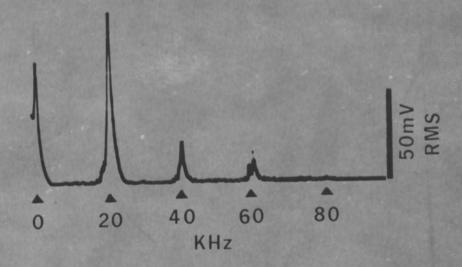
MIDDLE: Spectral analysis of the same sound made with a Tektronix 3L5 spectrum analyzer. The fundamental frequency is at 19.0 - 20.0 kilohertz (kHz). Two smaller harmonics appear at 40 kHz and 60 kHz.

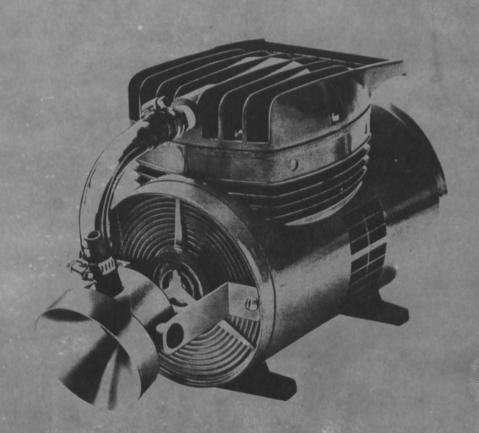
BELOW: Compressor and ultrasonic whistle.

Dimensions: Length - 30 cm

Width - 16 cm







CHAPTER I

The Effects of Natural and Man-made Ultrasound on Commensal Rodent Behaviour

INTRODUCTION

Acoustic communication has previously been thought to play only a small role in the social behaviour of commensal rodents, particularly rats (Barnett 1963; Busnel 1963). With the increasing sophistication of sound detecting and producing equipment in recent years however, evidence is being gathered which suggests that ultrasound may be an important factor in the social behaviour of many rodents.

Collias (1960) divides acoustic signals into ecological and functional categories related to: 1, predators; 2, sexual behaviour and related fighting; 3, parent-young relationships; 4, food; and 5, aggregations and group movements (the first three of these impinging on the present studies). The communicative value of any signal is determined through a change in or influence upon the behaviour of a second individual (Tembrock 1963). Both rat pups and mouse pups produce ultrasonic vocalization in response to: 1, isolation; 2, exposure to cold; 3, tactile stimuli; and 4, possibly hunger (Noirot 1966, 1968; Noirot and Pye 1969; Sewell 1967, 1969, 1970; Allin and Banks 1971). Okon (1970 a, b; 1972) found that these so-called "distress calls" change with the pups' ages and the degree of stimulation. Allin and Banks (1972) determined a

communicative value of rat pup distress calls through playback of recorded cries to adult rats. Lactating females were induced to leave the nest and engage in searching behaviour.

Sewell (1970) showed that ultrasonic distress calls of Apodemus sylvaticus serve a similar function. Vocalizations of both rat and mouse pups in response to tactile stimuli tend to be more intense and of longer duration than those emitted in response to isolation or cold. Noirot (1966) suggested that these loud calls may inhibit aggression of the mother towards the pup while being touched or carried.

Adult male rats during aggressive-submissive encounters produce two types of ultrasonic vocalization (Sales 1972 b). Short ultrasounds of 3 - 64 ms duration and at frequencies of about 50 kHz were produced by males in aggressive situations. while long pulses of 800 - 1600 ms duration at about 25 kHz were produced by males in submissive situations. Long pulses may serve to inhibit aggression or contact by the more dominant animal and short pulses may cause a less agressive rat to submit to a more aggressive animal with little or no conflict, although this effect is less clear. Barfield & Geyer (1972) reported long (1800 ms) vocalizations at 22 kHz from male albino rats during the post-ejaculatory refractory period (during which intercourse is not attempted). It was suggested that these vocalizations minimize contact of the female with the male. A variety of long and short ultrasounds are produced by male rats and mice during sexual activity (Sales 1972 a). Possible

communicative functions are unknown.

Campbell (1957) found that albino rats showed an aversion to sounds of lower intensities and frequencies than those needed to produce audiogenic seizures. Moreover, as the frequency increased, the threshold intensity for the aversive response was reduced. At 5 kHz, the threshold for this response was 80 Again with albino rats, non-damaging levels of white noise db. (composed of a broad spectrum of frequencies) are far less aversive than electric shock intensities typically used in psychological research, with 30 V equivalent to 85 db of white noise and 50 V equivalent to 120 db of white noise (Campbell and Bloom 1965). However, both studies employed sound largely in the sonic range and, as the auditory threshold for ultrasound is lower, ultrasound should be a more effective repellent. Burger (1967) found that 17 kHz sound at 100 db at 30 cm had moderate repellent qualities for several days. Sprock et al. (1967) described a variety of experiments with electronic equipment producing sound ranging from 4 - 19 kHz at 120 db with equivocal results. However, very few animals were used, some were reused, and these were not examined for hearing loss (or deafness) as experiments progressed. Greaves and Rowe (1969) reported success in preventing invasion of a new environment in a laboratory setting using wild rats. Ultrasound of 26 kHz at 120 db at 30 cm inhibited activity for 19 days. Mice were repelled for 23 days. In all these studies, it was suggested that habituation to the sound occurred.

Habituation to a stimulus which normally leads to a particular consummatory response without other stimuli being involved occurs less rapidly than to either a non-specific stimulus or to one that requires additional stimuli from other sensory modalities for consummation. Thus, habituation to a natural or stimulated bioacoustic signal with communicative value should be slower than to a signal requiring visual, tactile or olfactory cues to evoke a response. Also, if habituation to a bioacoustic signal did ultimately occur, the communicative value of the signal would be lost to the popula-Either situation might be exploited as management tech-The natural ultrasounds of rats and mice are relatively niques. pure tones with little frequency modulation and effectively elicit responses at intensities lower than synthetic ultrasounds They are also cheaper to produce and employed as repellents. easier to propagate, and theoretically more specific in effect than the synthetic repellents. However, little is known of the ability of bioacoustic signals to elicit responses from rodents independent of other cues.

Noirot (1968) suggested that the ultrasonic vocalizations of mouse pups have communicative value to lactating females and leads to searching behaviour. Initial support for this hypothesis comes from Brown's (1973 b) findings that the second high frequency peaks (40 and 50 kHz) in auditory thresholds of rats and mice respectively corresponds with the average frequency of the pups' vocalizations as well as some of the adult

vocalizations. The first part of this study compares the relative efficacy of recorded and live pup vocalizations in eliciting retrieval behaviour in lactating female mice. It was my purpose in the second part of this study to examine the acoustic signals of Rattus norvegicus, Rattus rattus and Mus musculus in response to electric shock and fear for purely ultrasonic cries. The possible intraspecific communicative value of ultrasonic vocalizations produced by stressed animals was considered.

The third and fourth parts of the study test the hypothesis that high intensity ultrasound has repellent effects on rat and mouse behaviour, and investigate the effects of different internal states and the availability of food and water on the animals responses to ultrasound.

Experiment 1

Ultrasonic Communication Between Mouse Pups and Lactating Females

Objective

The purpose of this experiment was to compare the effects of live and recorded mouse pup vocalizations on the pup retrieval behaviour of lactating <u>Mus musculus</u> females.

METHODS AND MATERIALS

Individually caged adult female multiparous white outbred mice were used in this study. At the time of testing, all the subjects were nursing litters of eight pups 3 - 5 days old. Isolated live 4-day-old mouse pups, recordings of distress cries of 4-day-old isolated mouse pups, and recordings of background noise related to the vocalization recording were presented as stimuli to lactating females.

To record ultrasounds, three 4-day-old pups from a litter of ten born to a multiparous female were placed separately on the floor of a pneumatically suspended chamber 91 cm x 91 cm x 91 cm in the box sound levels were down 12 db over the ambient level in the room and the temperature was 22° C. With the microphone held approximately 5 cm from the pup's mouth, five minute recordings of each pup's spontaneous vocalizations were made. Pups were not handled to elicit vocalizations. Sound spectro-

grams of selected recorded vocalizations were made and two of these are presented in Fig. 2. Most of the pulses or squeaks produced by the pups consisted of a fundamental at 55 - 65 kHz with occasional traces of a second harmonic at 110 - 130 kHz. Frequency modulation was limited to a slow drift down of 1 - 5 kHz from the start of the pulse. The average pulse length was 70 ms.

A 90-s recording with 102 of one pup's vocalizations was arbitrarily selected to be the experimental tape. Five live, spontaneously vocalizing 4 - 5 day old pups were used individually as the live experimental stimulus.

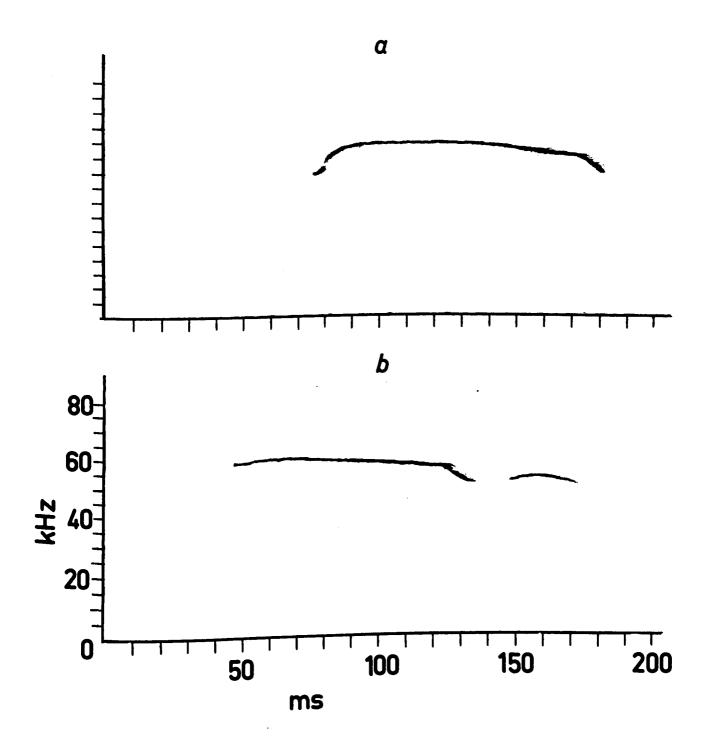
A 90-s recording of background noise from the recording chamber was designated as a control stimulus. Deafness in one of the groups of adult females was produced three days prior to the experiments through exposure to ultrasounds of 19 - 20 kHz at 140 db SPL at 30 cm for a period of 90 min. Deafness resulting from ruptured tympani was confirmed in these subjects from observations with an otoscope after lightly anaesthetizing the mice with ether.

The females, individually housed with their litters, were brought into the laboratory within hours of giving birth and their nest boxes positioned in the centre of the back wall of the cage with a space of 10 cm between the back of the nest box and cage. The transducer was placed in a corner in front of the nest box, approximately 20 cm from the entrance to the nest box. Tests were carried out between 1100 and 1400 hours. One group

Figure 2

Sonagrams of ultrasonic distress vocalizations from a 4-day-old albino mouse pup.

- A. Vocalization with a rapid frequency sweep at the beginning, slow downward drift, and rapid downwards sweep at the end.
- B. A slow frequency drift is followed by a rapid frequency sweep. The second vocalization is not a step, but a short, sweeping cry. On all sonagrams the vertical scale is frequency in kHz and the horizontal scale is time in msec. Darkness of the trace represents amplitude.



of females with intact hearing was given a stimulus of live pups, the second group was presented with the 90-s recording of vocalizations and the third was presented with the 90-s recording of background noise. The deafened females were presented with live pups. Each subject in each of the groups was given five trials with the same 90-s stimulus presented each time. Inter-trial intervals were 5 minutes. Positive responses or sorties were considered to be when a female made a complete exit from the nest box within the 90-s period the stimulus was presented, and oriented at least her head toward the stimulus. Live pups were transferred to the experimental cages in watch glasses to minimize possibilities of eliciting cries of a different nature from those on the recording.

RESULTS

The responses of females with intact hearing to live pups generally occurred within 15-s and in no case did they fail to find the pup within 15-s of leaving the nest box. After carrying pups into the nest, the females often made subsequent exits, or at least poked their heads out one or more times. The females presented with recording of pup distress vocalizations initially behaved in a similar manner, but never reappeared from the nest upon entering. These animals, in the first trials, often approached the transducer, sniffing at and around it. This behaviour quickly waned over subsequent trials. deafened females made no sorties from the nest box during the These animals fed normally and appeared to nurse their young although the survival of the litters to the age of weaning was less than half that of litters born and reared by The control animals made few responses and did normal females. not investigate the transducer. The results are summarized in Table 1. A Chi² test (Table 2) applied to responses of lactating females with normal hearing towards live pups and to recorded distress cries led to a rejection of the null hypothesis that there was no significant difference between the efficacy of the two stimuli in eliciting retrieval responses, i.e. females respond to live pups more than to recordings.

TABLE 1

Sorties by Lactating Mice in Response to Live Pups and Recorded Ultrasonic Distress Calls

	Stimulus: Live 4 day old pups	Stimulus:Record- ing of 4 day old pup	Stimulus:Record- ing of background noise	Stimulus:Live 4 day old pups
,	Subjects: 6 normal lactating females	Subjects: 6 normal lactating females	Subjects: 5 normal lactating females	Subjects: 5 deafened lactating females
trials	30	30	25	25
positive responses	27	. 15	α	0
% positive responses	06	50	ω	0

TABLE 2

A Test of the Hypothesis that There is no Significant Difference Between the Responses of Lactating Female Mice to Isolated Pups and Recorded Distress Cries of Isolated Pups.

	LIVE PUPS	UPS	RECORDINGS	INGS	TOTAL
	Observed	Expected	Observed	Expected	
Positive Responses	27	(21)	15	(21)	75
No response	K	(6)	15	(6)	18
TOTAL NUMBER OF TRIALS	30	30	30	30	09
$X_1^2 = \frac{(0-E)^2}{E} = 1.7142 + 1.7142$.7142 + 4.00 + 4.00	+ 4.00			
X_1^2 calculated = 11.4284					
X^2 (0.001) = 10.45					
: the null hypothesis is rejected	ejected				

Experiment 2

Stress Induced Ultrasonic Vocalizations from Commensal Rodents

Objective

The purpose of this experiment was to determine whether or not mildly-stressed R. norvegicus, R. rattus and M. musculus Would emit ultrasounds.

METHODS AND MATERIALS

Four adult male and four adult female wild Rattus

norvegicus, 4 - 6 months old; two wild-caught adult male

Rattus rattus, ages unknown; four juvenile male Rattus norvegicus,

32, 50, 74 and 87 days old; and four adult male and four adult

female wild Mus musculus, 3 - 5 months were used. All animals

were individually caged over the testing period.

Two methods were employed in attempts to elicit vocalizations from each subject. In the first, animals maintained 24 hours in the small cages were brought into the laboratory where they were stimulated visually by gesturing, and mechanically with a glass rod for a total of ten minutes. After a 24 hour recovery period, each subject was transferred to a foam rubber cylinder with a wire mesh floor. The dimensions of the cylinder were adjusted so as to restrict the animals' movements. Electric shocks of 100 V at a very low amperage were delivered through the mesh floor each second for a two minute period followed by a two minute recovery period. Five series of shocks were

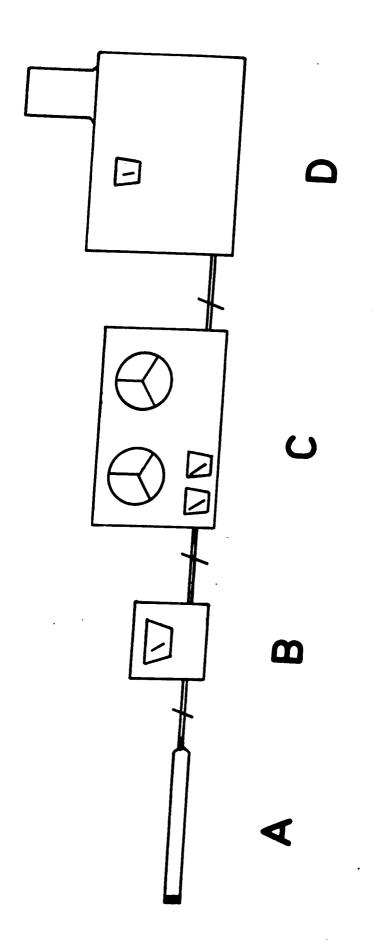
administered to each animal. Tape recordings for each subject from both methods were examined at 1/10th recording speed to detect ultrasonic vocalizations with the equipment shown in Fig. 3. Sonagrams of selected ultrasonic and audible vocalizations were made.

Figure 3

Apparatus for detecting, recording and analyzing ultrasonic vocalizations from rats and mice.

- A: Brüel and Kjaer condenser microphone.
- B: Hewlett Packard preamplifier.
- C: Precision Instruments tape recorder.

Signals were recorded at 37.5 i.p.s. and played back into the Kay Electric Co. Sona-Graph (D.) at 3.75 i.p.s. The equipment gave flat responses from 10 - 80 kHz.



RESULTS

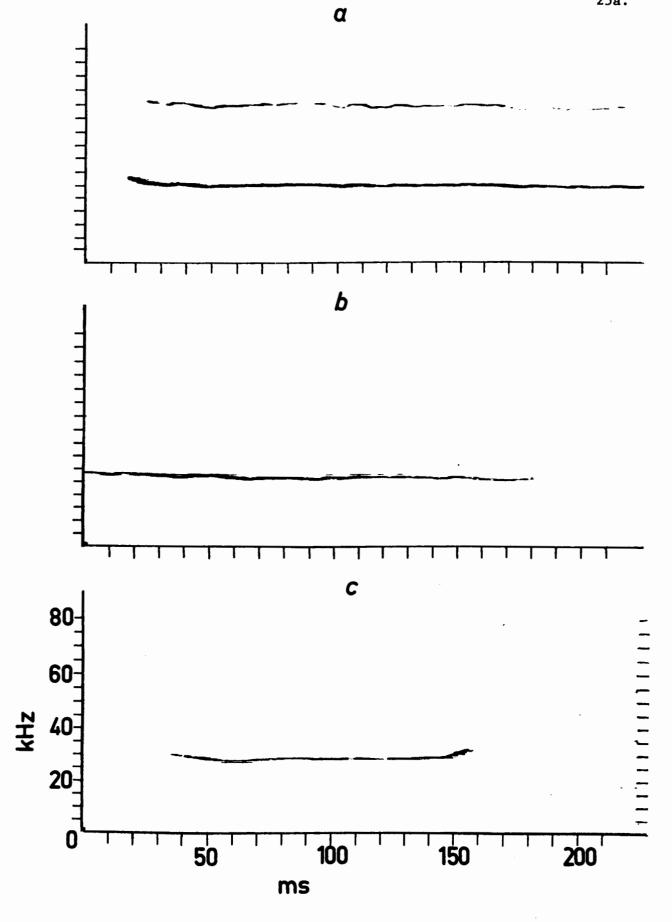
All animals were brought into a state of anxiety or fear as indicated by increased autonomic activation (urination, defaecation, increased heart rate, and breathing), with both forms of stimulation. Juvenile male Rattus norvegicus produced ultrasonic vocalizations in response to gestures and mechanical stimulation, but did not in response to electrical stimulation. Adult R. norvegicus, R. rattus, and Mus musculus did not produce any ultrasounds. Audible vocalizations were produced by all animals (adult and juvenile) as a result of both forms The ultrasonic squeaks of all four juveniles of stimulation. always followed the stimulus by one to several seconds, and were always associated with still, crouching posture. The ultrasonic vocalizations were similar in frequency and length. ever, substantial differences in other aspects of the cries appeared and were possibly related to the age of the animals. The vocalizations were emitted in trains consisting of 1 - 29squeaks with regular intervals of approximately 100 ms between Sonagrams of representative squeaks squeaks within a train. from an animal appear in Fig. 4. Most of the acoustic energy is located at 27 - 30 kHz, the fundamental frequency. A small second harmonic appears at 54 - 60 kHz. Modulation consists of a slight downward drift in frequency. In comparison to the other three, the youngest animal emitted more and longer trains, consisting of more squeaks, from the least amount of stimulation. With each of the older animals in turn, progressively fewer

Sonograms of ultrasonic vocalizations produced by juvenile \underline{R} . norvegicus while experimentally stressed.

- A: First portion of squeak from 50 day old male. Note harmonic at 60 kHz.
- B: End of squeak from 50 day old male.
- C: A short squeak from a 74 day old male. The vocalization was of lower intensity than A or B.

On all sonograms the vertical scale is frequency in kHz and the horizontal scale is time in msec.





trains composed of fewer squeaks were emitted, even under intense stimulation. The results are summarized in Table 3. These differences in vocalization quality and quantity appeared not to be the result of different levels of stimulation, as indicated by posture, grooming, defaecation and urination (Barnett 1963). The intensity of the vocalizations were not measured with the sound level meter. From the strength of the signal entering the preamplifier, the vocalizations were estimated to be 85 - 95 db at 10 cm.

The four juvenile rats were retested 40 days later and ultrasonic vocalizations could be elicited from only the two younger animals. As with the first series of tests, squeaks from the 90 day old animal were few and most difficult to elicit.

TABLE 3

Juvenile Male Rat Ultrasonic Vocalizations

	Rat 1 32 days old	Rat 2 50 days	Rat 3 74 days	Rat 4 87 days	1 & 2 & 3 & 4	F values cal- culated and expected*
Range of Fre- quencies of Vocalizations (kHz)	25 - 29 N = 70	25.5 - 29 N = 50	25 - 28 N = 30	25 - 28.5 N = 20	25 - 29 N = 170	
X Frequencies of Squeaks (kHz)	26.5 ± 0.2 N = 70	26.9 ± 0.1 N = 50	27.2 ± 0.2 N = 30	26.8 ± 0.3 N = 20	26.6 ± 8.9 N = 170	F = 1.7042 F(0.05)=2.6049
\overline{X} Length of Squeaks (ms)	406.5±16.0 N = 70	$427.2^{+}_{1}17.8$ $N = 50$	428.2 ⁺ 26.7 N = 30	422.8 [±] 25.1 N = 20	418.3 ⁺ 10.1 N = 170	F = 0.3308 F(0.05)=2.6049
X Intervals Between Squeaks	101.0±0.5 N = 50	$100.3^{+}_{-}0.3$ N = 40	100.0±0.4 N = 20	100.1 [±] 0.5 N = 14	100.5 ⁺ 0.2 N = 125	F = 0.8007 F(0.05)=2.6802
X Squeaks Per Train	10.6 ± 1.5 $N = 30$	7.0 ± 1.0 $N = 21$	3.8 ± 0.5 N = 17	2.3 + 1.2 N = 12		F = 7.990 F(0.05)=2.7581
$\overline{\mathrm{X}}$ Length of Trains of Squeaks (s)	4.6 ± 0.7 N = 30	3.9 ± 0.6 N = 21	1.6 ± 0.3 N = 17	1.0 + 0.2 N = 12		F = 7.3762 F(0.05)=2.7581
* from analvsi	from analysis of variance.	ഥ	distribution in D	Dixon and Massey.	1969, p.	470.

distribution in Dixon and Massey, 1909, p. 4/0. * from analysis of variance.

DISCUSSION

Recorded and live mouse pup distress vocalizations were effective in eliciting retrieval behaviour from lactating females with intact hearing. As such, the distress cries have a definite communicative value between mother and pup. However, from the results of this study, the hypotheses that vocalizations themselves could maintain retrieving activity had to be rejected. Habituation, a relatively permanent waning of responses resulting from unreinforced stimuli (Thorpe 1963), occurred after several positive responses towards the recorded vocalizations. Noirot (1965) elicited retrieval responses from lactating females towards live young 148 times in succession with no habituation observed. Casual observation in this study indicated that recordings did not lose their effect if the female was "rewarded" with a pup for approaching the transducer. than the recordings being of poor fidelity, possibly more than auditory stimulation is necessary to maintain retrieval behaviour. Indirect evidence that olfactory cues are involved comes from a study by Beach and Jaynes (1956) where a difference in retrieval of their own and other young disappeared in lactating rats with cauterization of olfactory bulbs. Visual and tactile cues may also be necessary to complete the retrieval response and to prime subsequent searching behaviour often seen after a female has made a successful retrieval but not seen in females presented with only recordings. The positive responses of lactating females towards distress vocalizations are generalized and nonselective. The speed of the female's response and the relative richness of odours in the nest lead to the conclusion that in this study odours from the isolated pup did not initiate retrie'val responses.

The survival value of both the ability to perceive and emit ultrasound is apparent. Pups, blind and helpless for at least 10 days, are often dragged from the nest on the mother's nipples. Young of deafened mothers must rely on chance that other of her sensory modalities will lead to retrieval. Brown (1973 b) reports that for both rats and mice, the peak in auditory sensitivity at high frequencies (ultrasonic) is enhanced at the level of the inferior colliculus. These enhanced peaks in both Rattus and Mus occur at frequencies of pup and some adult vocalizations. Noirot (1966) suggests some pup vocalizations inhibit aggression from the mother. The effect of deafening on the survival rates of litters owing to both the loss of retrieval behaviour and possible maternal aggression needs further exploration.

Juvenile male rats readily emit trains of long ultrasonic pulses when stressed. These vocalizations may be analogous to those reported by Sewell (1969) and Sales (1972a) from submissive male rats in responses to advances and threats by dominant males. Sales (1972 a) suggested that the cries serve to minimize contact. However, the cries she reported were more than twice as long (800 - 1600 ms) than the ones reported here and were not emitted in long trains, rather in one to three cries

in a train or sequence. Barfield (1972) reported long (800 ms) 22 kHz post ejaculatory vocalization emitted by males in long trains similar to those described in this study. Intervals between pulses or cries were also longer (250 ms). It was suggested that these vocalizations inhibit contact from the female. The distress cries of rat pups commonly have a frequency of 30 - 40 kHz (Allin and Banks 1972).

The results of this study suggest a progression with age of the animals for the length of trains of cries, but not for the frequency, length of cries, or interval between cries. However, more individuals in each age group should be tested to find if these progressions are real. In all cases, cries were associated with a still crouching posture, and a slight lifting of the head. Sales (1972 a) reports similar observations. It is unknown whether the cries are whistles, are produced in the larynx, or are the result of modifications of the superior thyro-arytenoid folds located in the trachea above the larynx and below the epiglottis (Roberts 1972).

In the social structure of wild rats, young males are generally of lower "status" than mature males (Barnett 1963), and the association of more acoustic energy (intensity x duration) with younger animals may reflect the value of the cries in inhibiting aggression towards immature males in particular or submissive ones in general. It is unknown whether juvenile females produce similar vocalizations when stressed.

The role of ultrasonic cries of juvenile males in the social

behaviour of rats needs to be determined more precisely. The potential of these or similar vocalizations for control purposes should be explored. There appears to be a potential value in the distress vocalizations of mouse pups as a short term attractant. Rat pup distress vocalizations, likewise could serve as attractants in population surveys or in integrated control programmes.

Experiment 3

Immediate Effects of High Intensity Ultrasound on Rats

Objective |

The purpose of this experiment was to test the immediate repellent effects of high intensity ultrasound on adult \underline{R} .

Norvegicus and \underline{R} . rattus.

METHODS AND MATERIALS

Fifteen wild adult male and female \underline{Rattus} $\underline{norvegicus}$ and three wild adult male \underline{Rattus} were used in this experiment.

The experimental apparatus consisted of a U-shaped run 4 m long, 0.7 m high, 0.7 m wide. The outside and ends of the U were of galvanised sheet metal, the inside face of the U was of clear plexiglass and the top and bottom were of 1.27 cm wire mesh.

With an air driven ultrasonic whistle (described in General Methods) suspended 1 m above the floor at the end of the U, the sound level at the opposite end of the U was raised 14 db above the ambient level of 70 db SPL to 84 db. The electrically driven air compressor, isolated on foam rubber pads and positioned 8 m from the run, raised the ambient sound level 3 db. All sound pressures were measured with the B and K Sound Level Meter. Lighting was supplied by four 100-W incandescent bulbs suspended above the run.

The rats were introduced singly into the run and allowed

to explore for 30 min. At the end of this period, the rat was invariably in one of the ends of the U, probably because it afforded cover on two sides. The whistle was suspended always over the end with the rat and switched on for a 30-s period. The animals' responses were observed and recorded.

Five adult Rattus norvegicus were exposed to noise from the compressor suspended over one of the two end boxes in a manner similar to that previously outlined. This sound consisted of low frequency noise at a level of 88 db SPL at 1 m.

RESULTS

Within 20-s every rat had run to the opposite end box in the run and had assumed a crouching posture with ears flattened. All rats of both species exhibited increased urination, defaecation, followed by intense grooming activity and abrupt movements with bodies flattened against the cage floor. Four animals, with the onset of the ultrasound, leapt at the roof of the cage and thrashed wildly about the sound box before running to the other end of the run. In contrast, the compressor noise elicited a small jump from three individuals and ear twitches in two. One animal trotted into the other end box, but returned immediately.

Experiment 4

Effects of High Intensity Ultrasound on the Behaviour of Rats: Part 1

Objective

Changes in the repellent effects of high intensity ultrasound over a three-day period on \underline{R} . norvegicus given a single source of food and water in the sound field were measured in this experiment.

METHODS AND MATERIALS

Nine wild adult male and female Rattus norvegicus were used in this study.

A V-shaped run with two end boxes 0.7 m x 0.7 m was constructed for this experiment. Three sides of the end boxes were of galvanized sheet metal, the inside face was of clear Plexiglass and the top and bottom were of 1.27 cm metal mesh. The apex of the V was a sheet metal 90° elbow 20 cm in diameter. Two 1 m cylinders of 1.27 cm mesh and 20 cm in diameter connected the elbow to each end box. A photoelectric cell illuminated by a red light was placed across the entrance to one end box designated the experimental box. A super 8 mm movie camera in front of the experimental cage was switched by the photoelectric cell through a timer to run 7-s each time the beam was tripped. One 15.2 m Tri-X movie film was placed in the camera each day of the experiment. A clock was placed near the

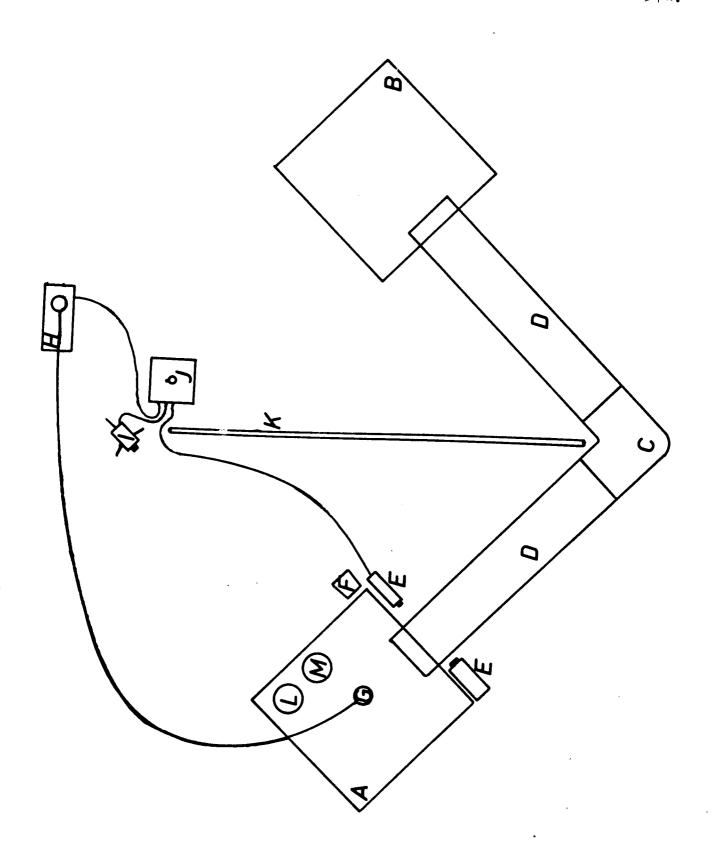
experimental cage within the field of the camera. The ultrasonic whistle, producing 130 db SPL at 1 m was suspended over the experimental box. With the whistle operating, the sound level in the elbow was 80 db, 10 db above ambient, and the level in the control box was 78 db, 8 db above ambient. Compressor noise raised the ambient level 3 db. Continuous lighting was supplied by 4 100-W incandescent bulbs. See Fig. 5.

Rats were introduced singly, and after a three day acclimation period, activity was monitored by the camera for a three-day period. The whistle was then switched to operate in synchrony with the camera, i.e. for a minimum seven second period each time the photo-beam was tripped by a rat entering or leaving the experimental box. The whistle and camera operated for as long as the animal remained in the entrance to the experimental box. After a three-day experimental period, the whistle was disconnected and activity was filmed for a further three-day period. Food and water were replenished daily between 0830 and 1030 hours in the experimental box only.

Filmed observations from each day of the experiments were projected at 5 frames per second, effectively slowing action to 0.21 normal. The time of each animal's visit to the experimental box, as well as its length and resulting exposure of the rat to the film or film plus ultrasound were recorded. Comments on the animals' general behaviour, responses to the ultrasound and feeding behaviour were noted. Data from films covering less

Experimental apparatus for measuring repellent effects of high intensity ultrasound on rats. Looking down from above:

- A: Experimental end box
- B: Control end box
- C: Sheet metal elbow
- D: Wire mesh tunnels
- E: Photoelectric cell
- F: Clock
- G: Ultrasonic whistle suspended from above
- H: Air compressor supplying whistle
- I: Movie camera on a tripod
- J: Timer
- K: Ultrasound proof barrier
- L: Food dish
- M: Water dish



than one hour was not used. There were no partially exposed films, i.e. all films monitored less than a rat's full day's activity.

Experiment 5

Effects of High Intensity Ultrasound on the Behaviour of Rats: Part 2

Objective

Changes in the repellent effects of high intensity ultrasound over a three day period on \underline{R} . norvegicus given an alternative source of food and water outside the sound-field were measured in the experiment.

METHODS AND MATERIALS

Eight wild adult male and female Rattus norvegicus were used in this study. These individuals were different from those used in previous studies.

Equipment and procedure were as in 4 except that food and water were placed in the end boxes at each end of the run.

RESULTS

In all cases, the animals made nests from Dacron fibre placed in the elbow of the tunnel connecting the two end boxes. Presumably as a result of the continuous illumination, activity was not restricted to evening hours. There was a great variability among animals as to how food was taken. Several hoarded the pellets, making successive trips to the food dish until it was emptied. This food was often hoarded in the nest. Other animals removed one pellet periodically and never emptied the dish, while only two of the seventeen animals in these two experiments ate in the experimental area. One animal, an adolescent male (90 - 100 days), began hoarding on the third day of exposure to ultrasound and continued for the duration of the experiment.

As in experiment 3, the first response of the rats to the ultrasound was a pronounced jump followed by escape from the soundfield.

In both experiments 4 and 5 (Fig. 6 a), the ultrasound significantly reduced the average amount of time, measured in seconds per hour over three-day periods spent in the sound field. These and other values from both experiments are summarized in Table 4.

In experiment 4, animals spent less time sitting in the entrance of the experimental cage tripping the photocell when the whistle was operating than in either the sound free periods preceding or following (Fig. 6 b). With the whistle operating,

- a. The amount of time measured in seconds per hour that rats spent in the ultrasound field (19 20 kHz at 130 db).
- b. The amount of time measured in seconds per visit that rats spent in the ultrasound field.

In both a and b:



= rats with a single source of food and water in the experimental cage (Experiment 4)



= rats with food and water in both the experimental and in the control cage (Experiment 5)

= the three day period when the ultrasonic whistle was operating.

Rats sitting in the entrance were counted as being in the ultrasound field.

In b, data from Experiment 5 was not significant.

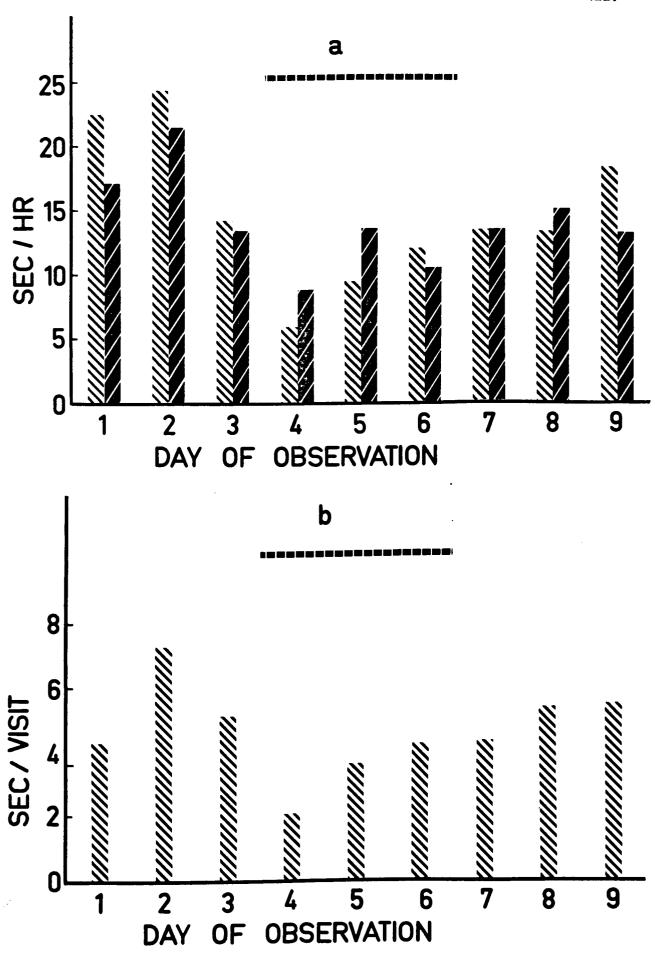


TABLE 4

Ultrasound was Presented in Periods B and B' Rat Activity in the Experimental Cage in Experiments 4 and 5.

	∵	Experiment 4 (means ± S.E.)		H)	Experiment 5 means ± S.E.)		Comparisons of Means	of Means*
PARAMETERS MEASURED	A - 1 - 5 - 5 - 5 - 5 - 5 - 5 - 5 - 5 - 5	Period B 4 - 6	6 - 2	A' 1 - 3	Period Bi 4 - 6	6 7	Within Experiment	Between Experiments
Mean Amount of Time Per Hour in Sound Field (s)	20.3 ± 2.6 N = 129	8.8 ± 0.8 N = 235	13.9 ± 1.4 N = 158	16.7 ± 2.2 N = 143	10.7 ± 1.2 N = 205	13.9 ± 1.7 N = 178	A>B, P<0.01 A>C, P<0.05 C>B, P<0.01 A'>B', P<0.01	
Mean Amount of Time Per Visit in Sound Field (s)	5.6 ± 0.6 N = 129	3.2 ± 0.2 N = 235	4.9 ± 0.4 N = 158	4.4 ± 0.4 N = 143	3.8 ± 0.3 N = 205	4.1 ± 0.3 N = 178	A>B, P<0.01 C>B, P<0.01	
Total Amount of Time in Experimental Area (% of Observation Period)	4.9 ⁺ 1.0 N = 129	4 0.X	7.7 ± 1.2 N = 158	4.2 ± 0.7 N = 143	2.5 ± 0.5 N = 205	7.7 8.11 1.11	C>B, P<0.01 A'>B', P<0.05 C'>B', P<0.01	
Frequency of Visits Per Hour to Take Food	0.6 ± 0.1 N = 129	o N N	0.6 ± 0.1 N = 158	0.4 ± 0.1 N = 143	0.1 ± 0.0 N = 205	0.2 ± 0.0 N = 178	A>B, P<0.05 C>B, P<0.05 A'>B', P<0.05	B>B', P<0.05 C>C', P<0.01
Frequency of Visits Per Hour to Take Water	0.6 ± 0.1 N = 129	0.4 ± 0.1 N = 235	0.6 ± 0.1 N = 158	0.2 ± 0.0 $N = 143$	0.1 ± 0.0 N = 205	0.3 ± 0.0 N = 178	B>C, P<0.05 A'>B', P<0.05 C'>B', P<0.01	A>A', P<0.01 B>B', P<0.01 C>C', P<0.01
Length of Visits to Experimental Area (s)	77.8 ± 28.4 N = 129	77.8 ± 28.4 83.9 ± 18.8 N = 129 N = 235	204.8 ± 47.4 N = 158	54.6 ± 10.1 N = 143	32.4 ± 5.9	95.8 ± 24.7	C>A, P <o.05 C>B, P<o.01 A'>B', P<o.05 C'>B', P<o.01< td=""><td>B>B', P<0.05 C>C', P<0.05</td></o.01<></o.05 </o.01 </o.05 	B>B', P<0.05 C>C', P<0.05

• Comparisons based on a one way analysis of variance with values of F converted to probabilities, P = 0.05 and P = 0.01, Dixon and Massey, pp. 470, 471.

in comparison with the preceding three-day period,
the total amount of time in the experimental box was significantly
less in experiment 5, where alternative sources of food and water
were available, but not in experiment 4 with no such choice (Fig.
7 a). There were fewer visits to the experimental area for food
with the whistle operating in comparison with the preceding
period. This was true for animals with alternative and single
sources of food (Figs. 8 a,b). However, in compensation, more
visits for food were made in 4 after the whistle was switched
off. This was not so in 5.

Also, when the results from the two experiments are compared, significant differences appear. While there were no differences in feeding behaviour, measured by visits for food and for water, in Period A of 4 and 5, fewer visits per hour for both food and water were made in Period B'in 5 than in Period B in 4.

Unlike animals in 4, animals with alternative sources of food and water did not resume feeding and taking water from the experimental area after the whistle had been switched off, indicating that a feeding pattern had been at least temporarily altered.

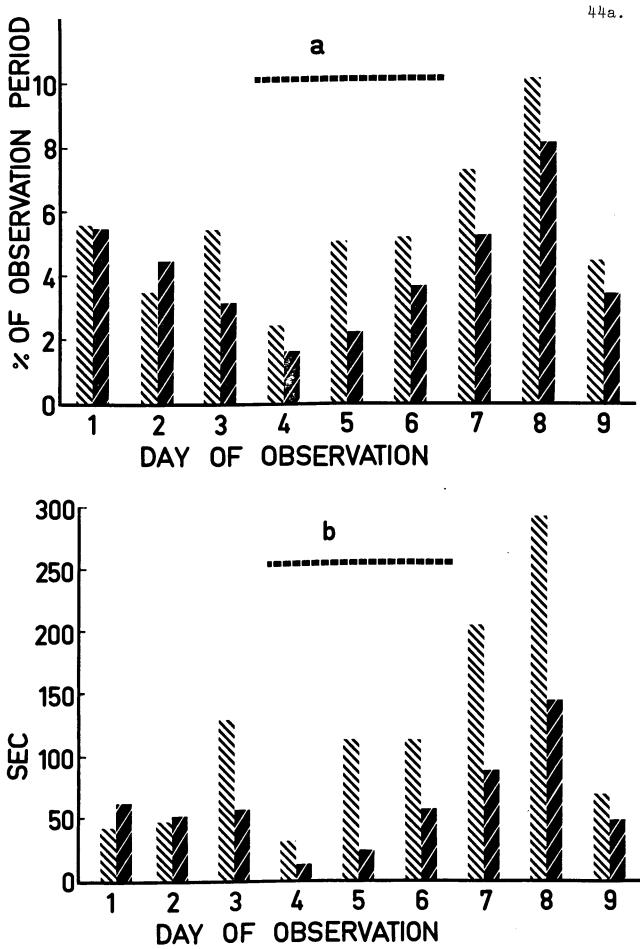
Another difference in response to ultrasound appears when lengths of each visit to the experimental area are compared between 4 and 5 (Fig. 7 b). Whereas there were no differences for Periods A and A', there was a difference while the whistle was operated (Periods B and B') and for the period following. Visits were shorter in 5 for both B' and C'. Data from experi-

- a. Total amount of time that rats spent in the experimental cage (% of observation period)
- b. Duration of visits made by rats to the experimental cage (measured in seconds per visit)

In both a and b:

- = rats with a single source of food and water in the experimental cage (Experiment 4)
- = rats with food and water both in the experimental and in the control cage (Experiment 5)
- was operating (19 20 kHz at 130 db).





Frequency of visits made by rats to the experimental cage.

- a. Rats with a single source of food and water in the experimental cage (Experiment 4).
- b. Rats with food and water in both the experimental and control cages (Experiment 5).

In both a and b:



= visits per hour for food



= visits per hour where neither food nor water were
taken

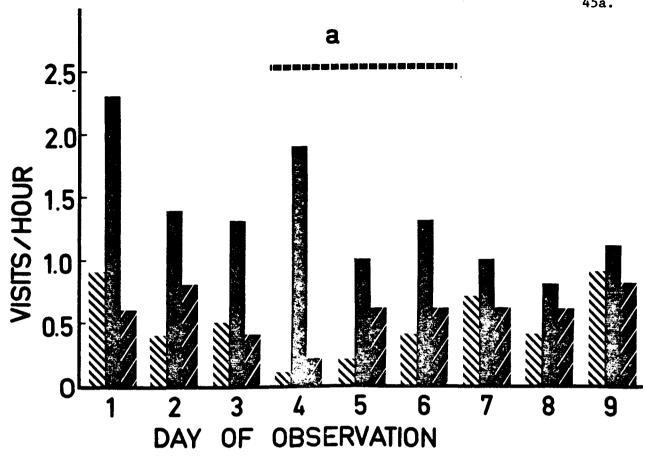


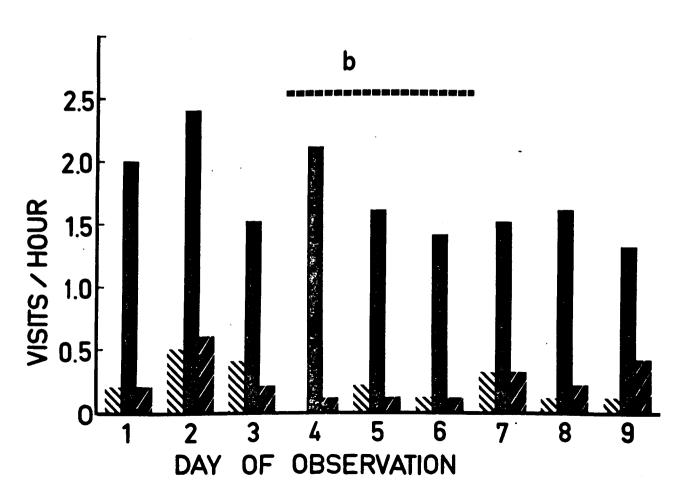
= visits per hour for water



= the three day period when the ultrasonic whistle was operating.







ments 4, 5, 6 appears in Appendix I.

When in the ultrasonic field, all animals moved about more quickly with ears flattened against their heads. There was a greater tendency to climb the cage and jump at the roof. For the first few visits in the final three day period with the whistle silent, many animals appeared hesitant, often remaining in the entrance. By the third day in Periods C and C', animals appeared less stressed and moved more slowly.

Hearing of the rats was qualitatively tested using a small piece of spring steel which produced a loud snap (100 db SPL) when deformed. Decisions on hearing activity were made observing Preyer's reflex (flattening of pinnae), movements of vibrissae and the generalized startle, or leap-crouch, response (Scheidt 1952). Seven of nine rats in experiment 5 gave reduced responses. Two rats from 4 and two from 5 with hearing losses were examined with an otoscope while lightly anaesthetized with ether. All had inflammation or haematomas of the tympani in comparison with controls. In all cases, hearing recovered within four weeks.

Experiment 6

Effects of High Intensity Ultrasound on the Behaviour of Rats: Part 3

Objective

Changes in the repellent effects of intermittent intervals of ultrasound over a 3 day period on groups of rats given a single source of food in the sound-field were measured in this experiment.

METHODS AND MATERIALS

Nine wild adult male and female <u>Rattus norvegicus</u> were used in this study. These rats were different from those used in previous studies.

Equipment and procedures were similar to those in 4 except for these changes: 1. Rats were introduced in groups of three into the apparatus; 2. the whistle, rather than being switched by the photocell, was switched by a specially fabricated timer at random intervals. Intervals of silence were from 15-s to 180-s duration and intervals of sound were of 3-s to 90-s duration. The camera was, as in both 4 and 5, switched by the photocell. On some occasions, two films were exposed on one day. White paint on the head was used to identify each rat. Data from each rat's visiting the experimental area was considered separately and averaged later on an hourly basis. Overlapping visits by two or more animals were measured separately.

RESULTS

The results for this experiment have been considered separately because of the difference in procedure. In addition to the ultrasound being presented in randomly assigned intervals, individual behaviour may have been influenced through observation of others. When Period A is compared with Period B, high intensity ultrasound did not significantly reduce the total amount of time spent in the experimental area, the averagle amount of time per hour or the average amount of time per visit (Figs. 9 a, b, c).

However, the behaviour of the rats as measured by these parameters in the three day period following ultrasound was significantly different compared with their behaviour with the whistle on. These differences are summarized in Table 5.

Although the difference is not significant, it was observed that less food and water were taken in Period B than in A or C.

Four of the nine rats when examined later appeared to have suffered hearing loss with associated inflammation of the tympani.

- a. The total amount of time that rats spent in the experimental cage (% of observation period)
- b. The amount of time measured in seconds per hour that rats spent in the ultrasound field
- c. The amount of time measured in seconds per visit that rats spent in the ultrasound field.

In a, b and c:



- = rats with a single source of food and water in the experimental area. Rats were introduced into the experimental apparatus in groups of three.
- = the three day period when ultrasound was presented in intervals of random duration.

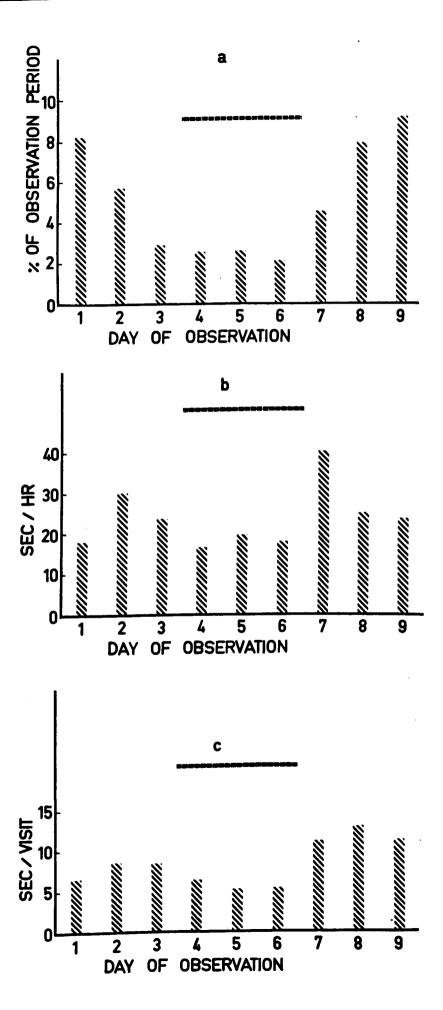


TABLE 5

Rat Activity in the Experimental Cage in Experiment 6. Animals were run in Groups of Three, Food and Water were Presented in Experimental Area, and Ultrasound was Presented for Randomly Determined Intervals in Period B.

	Exper	Experiment 6 (Means + S.E.)	S.E.)	
PARAMETERS MEASURED	PERIOD A (DAYS 1-3)	PERIOD B (DAYS 4-6)	PERIOD C (DAYS 7-9)	COMPARISON OF MEANS
Average amount of time per hour in sound field (s)	22.5 ± 3.2 N = 94	17.4 ± 2.7 N = 88	28.1 ± 4.4 N = 48	C> B, P < 0.05
Average amount of time per visit in sound field (s)	7.4 ± 0.9 N = 94	5.7 ± 0.8 N = 88	11.6 ± 2.4 N = 48	C> B, P < 0.01
Total amount of time in experimental area (% of observation period)	6.4 ± 2.0 N = 94	2.3 ± 0.8 N = 88	7.5 + 2.9 N = 48	C> B, P < 0.05

DISCUSSION

The repellent effects of high intensity ultrasound on both species of <u>Rattus</u> were clearly established from the first experiment in this series. Although the animals were tested 30 minutes after being introduced, the minimal responses to compressor noise would suggest the rats were relatively unstressed.

The reduced activity in the experimental area while the whistle was operated in experiment 4 provides evidence for the repellent effect of the ultrasonic field. This conclusion is further supported by the observed increase in activity after the Ultrasound was even more effective whistle was switched off. in reducing activity when an alternative source of food and water in a sound-free area was supplied (Exp. 5). The repellent effects of the whistle appeared to be instrumental in changing the feeding behaviour of rats offered a choice. Little food was taken from the control area prior to the whistle's operations, but for the remaining six days, little was taken from the experi-It is unknown how long this effect might last mental area. after cessation of the whistle's operation. When no alternative food and water source was offered, the repellent effect on feeding behaviour was less, with compensatory feeding in the period following the whistle's operation.

Neophobia, or the avoidance of a new object is a behaviour characteristic of wild <u>Rattus norvegicus</u> (Barnett 1963) and occurs when there is a change in an otherwise familiar situation. It may be evoked by an unfamiliar object, a familiar object in a new

position, or by unfamiliar noises. Although temporary, the duration of the effect is highly variable, ranging from hours to weeks (commonly hours to days). Upon removal of the new object, behaviour immediately returns to normal. Initially in both experiments, the repellent effects of the ultrasound were most likely strengthened by this neophobic effect. However, the overcompensatory activity in both experiments following the ultrasound and the reduced feeding activity in the experimental area in experiment 5, where alternative sources of food and water were available, are uncharacteristic of the neophobic effect.

More than one process may be involved in the waning of responsiveness towards ultrasound. Different aspects of the overall response wane at different rates. Even though animals withdrew from the soundfield less and less rapidly with repeated exposure, responses towards the sound itself persisted. These include rapid movement, flattening of the ears, climbing the cage and displacement grooming. Melzack (1961) found that ducklings still oriented towards hawk shaped figures even after escape responses habituated, while Horridge (1959) noted that Nereis retained some elements of withdrawal responses after habituation of other elements.

Ultrasound presented in randomly assigned intervals had little repellent effect. Some effect is evident from the changes in activity following the employment of ultrasound, but the hypothesis that irregularly occurring intervals of ultrasound inhi-

bit habituation must be rejected. Possibly the overall pattern or envelope of sounds and silence was recognized and habituation occurred. Conversely, because of the irregular occurrence of ultrasound, association between ultrasound and experimental area may not have been made. The latter is more likely, for when the whistle switched on with an animal in the experimental area, the rat invariably left within a fraction of a second. However, rats did enter the area with the whistle operating.

Testing three rats at one time may have possibly led to different patterns of behaviour from those seen in individually tested rats. Changes in behaviour resulting from the presence of another member of the same species, or social facilitation. (Crawford 1939) has been found to be of minimal importance in learning and general behaviour of rats (Holder 1958). However, this work was done with albino or other laboratory varieties whose social behaviour is much less rich than that of wild varieties (Barnett 1957, 1960, 1963). Leadership of a dominant rat may influence behaviour through imitation as well as the tendency to remain together. Observational learning, a particular form of social facilitation, has been reported in rats avoiding candle flames after observing others burned (Lore et al. 1971). Rats and mice can discriminate between the odours of stressed and nonstressed individuals (Valenta et al. 1968; Carr et al. 1970). Both the mechanism for these responses and their functional importance to overall behaviour are unknown. This relatively unexplored area of behaviour impinges directly

on successes and failure of control campaigns against commensal rodents, particularly trapping and poisoning campaigns.

Experiment 7

Repellent Effects of High Intensity Ultrasound on Mus musculus

Objective

The purpose of this series of experiments was to compare the repellent effects of ultrasound on mice in experimental situations where: (1) positioning of food sources was altered, (2) the subjects were deprived of food and water prior to the experiment, (3) the experimental area (sound field) was new to the subjects, (4) combinations of the above three. It was hypothesized that changes in the internal states of the animals would affect responses to the ultrasound.

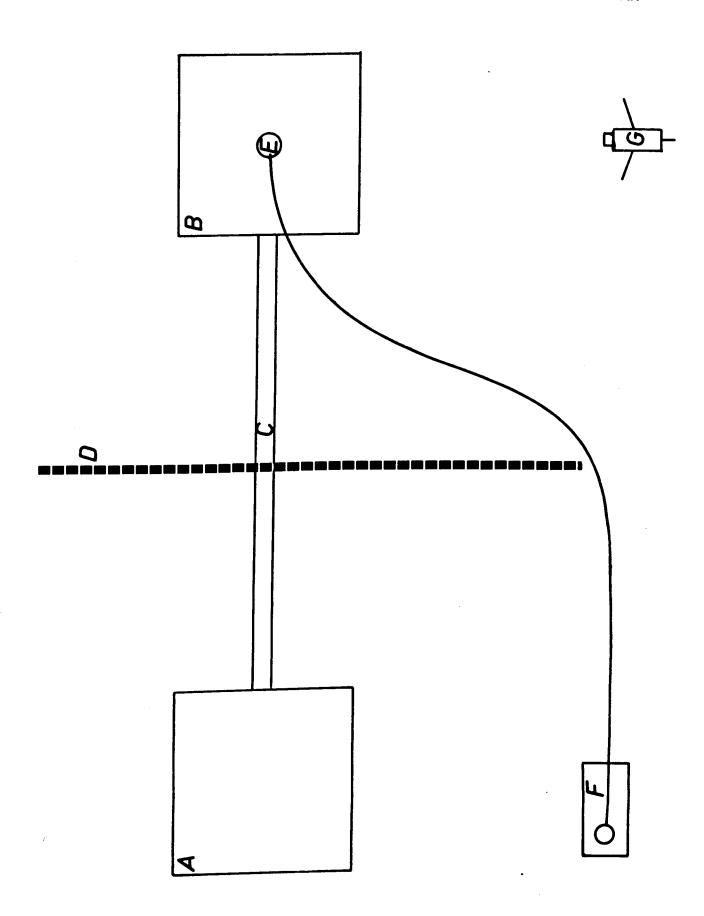
METHODS AND MATERIALS

Adult male and female wild $\underline{\text{Mus}}$ $\underline{\text{musculus}}$ were used in this series of ten experiments.

The testing apparatus consisted of two square plate glass cages 20 cm on a side with a 0.95 cm galvanized square mesh top. A cylinder of the same mesh, 50 cm long and 4 cm in diameter connected the two cages through 2.5 cm holes in a side of each cage. A baffle, 0.7 m x 0.7 m, constructed of acoustic tile, with a hole cut to accommodate the tunnel was positioned halfway between the two cages. An ultrasonic whistle (described in General Methods), detuned to produce only 120 db SPL at 40 cm, was suspended 40 cm above the floor of one of the two cages designated the experimental cage. The compressor, isolated on foam rubber pads, was positioned 2.4 m from the cages (Fig. 10). With the

Diagram of experimental apparatus used to measure the repellent effects of high intensity ultrasound on mice.

- A: Control area
- B: Experimental area
- C: Connecting tunnel (mesh)
- D: Ultrasound proof baffle
- E: Ultrasonic whistle suspended
- F: Air compressor for whistle
- G: Movie camera



whistle operating continuously, sound levels in the section of the tunnel adjacent to the experimental cage were 107 db, 88 db in the section adjacent to the control cage, and 86 db in the control cage. With only the compressor operating, sound levels in the tunnel and in both cages were up 2 db over the ambient level of 82 db SPL. Continuous lighting was supplied by four 100 Watt incandescent bulbs. A Nizo Super 8 mm movie camera exposing one frame every 5 seconds monitored activity in the experimental area.

Groups of six mice, all of the same sex and cage-mates for a minimum of four weeks were introduced into the experimental apparatus 24 hours prior to being tested. Experiments, each of 5 hours duration and started between 0900 - 1200 hours, were carried out over a 14 month period. Allowances were not made for possible shifts in activity related to circadian rhythms. A minimum of four replicates were run for each experiment, each using six mice with no previous experience of the experimental apparatus.

In the first series of experiments in the study, mice were allowed free access to both sides of the apparatus for both the acclimation and experimental period. Food and water were supplied ad lib. Mice in the second series were allowed free access to both sides of the apparatus, but were deprived of food and water during the 24 hr. acclimation period. In the third series, mice were restricted to the left (control) side of the apparatus and the tunnel during the acclimation period, but

were given food and water <u>ad lib</u>. In the fourth series, mice were deprived of both food and water and restricted to the control cage and tunnel during the acclimation period. The individual experiments are listed below:

i. Food and water:

- a. Present in control area.
 - 1. No ultrasound (4 replicates)
 - 2. With ultrasound (4 replicates)
- b. Present in both control and experimental areas.
 - 1. No ultrasound (8 replicates)
 - 2. With ultrasound (9 replicates)
- c. Present in experimental area.
 - 1. No ultrasound (6 replicates)
 - 2. With ultrasound (5 replicates)
- d. Absent in both areas
 - 1. No ultrasound (4 replicates)
 - 2. With ultrasound (4 replicates)
- ii. Deprivation (24 hours) followed by food and water:
 - a. Present in control area.
 - 1. No ultrasound (4 replicates)
 - 2. With ultrasound (4 replicates)
 - b. Present in experimental area.
 - 1. No ultrasound (4 replicates)
 - 2. With ultrasound (4 replicates)
 - c. Absent in both areas.
 - 1. No ultrasound (5 replicates)
 - 2. With ultrasound (4 replicates)
- iii. Opportunity to explore experimental area with food and water:
 - a. Present in both control and experimental areas
 - 1. No ultrasound (4 replicates)
 - 2. With ultrasound (4 replicates)
 - b. Present in experimental area.
 - 1. No ultrasound (4 replicates)
 - 2. With ultrasound (4 replicates)

- Opportunity to explore experimental area after food and iv. water deprivation (24 hours):
 - Food and water offered in experimental area.

 - No ultrasound (4 replicates)
 With ultrasound (5 replicates)

Exploded mica (Vermiculite) to a depth of 1 cm covered the floor of each cage. Cages were washed with detergent and dried between experiments to minimize the effect of odour of conspecifics. Food consisted of ground Purina Mouse Chow and was presented in the middle of the cage in 4 cm plastic petri-One ounce cylindrical open mouthed water jars were dishes. placed also in the centre of the cage. Identification from the film of individually marked mice was not possible.

Films of each experiment were projected at a rate of 5 frames per second, or when necessary, were viewed frame by Movements of individual mice into and out of the experiframe. mental area were recorded. Only if a mouse's head and at least two feet were in the experimental cage was it scored. mice entering and leaving were scored as 4 movements. could possibly enter and leave within the 5 second interval between frames being exposed and thus would not be scored. Data, upon which all further calculations were based, were collated into ten minute periods, for a total of thirty periods for each 5 hour experiment. A summary of means (with their standard errors) of movements into and out of the experimental area appears in Appendix II. Using a three point moving average, means for each experiment were plotted to show trends in changes

of activity over time. A measure of ultrasound's effects on activity was achieved by comparing at hourly intervals the numbers of movements into and out of the experimental area with the whistle on and off.

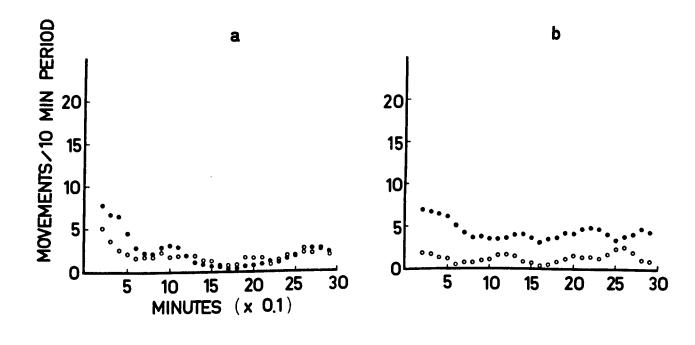
RESULTS

In the first series of experiments, i a-d (Figs. 11 a-d), the animals were familiar with the experimental area and were provided with ad lib food and water in the controlea (ia), in both areas (ib), in the experimental area (ic), or the food and water were removed just before the start of the experiment (id). The levels of activity and amplitude of change in activity as measured by movements in experiments i a-c were relatively low. With food and water in the control side only, levels of activity were the lowest of the four experiments. Experiment id represents a special case and would perhaps be better compared with itia and itib as the environment in the experimental area had been changed with the removal of the food and water dishes.

Ultrasound reduced activity to the lowest absolute levels in experiments ib and ic, although there is also a significant reduction in id. Significant reductions in activity are summarized on an hourly basis for all experiments in Table 6.

Data from these comparisons appear in appendix III.

In the second series of experiments, iia-c (Figs. 12 a-c), mice were familiar with both sides of the apparatus, but were deprived of food and water for 24 hours prior to the start of the experiments. Food and water were then placed in the control area (iia) or in the experimental area (iib). No food or water was provided in iic. With the whistle not operating, levels of activity are higher than in the first series, except for id. This activity gradually declines over the five-hour period, but least



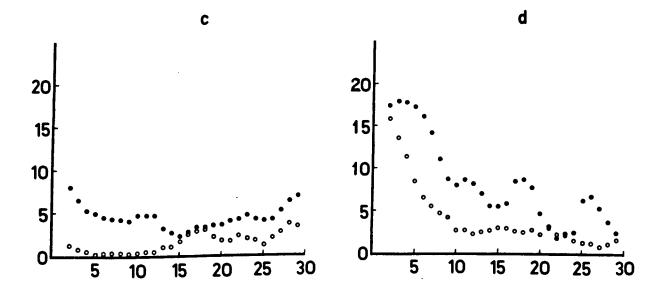


TABLE 6

Repellent Effects of High Intensity Ultrasound on Mouse Activity as Measured Through a Comparison of Movements into the Experimental Area with Ultrasound on and with it off.

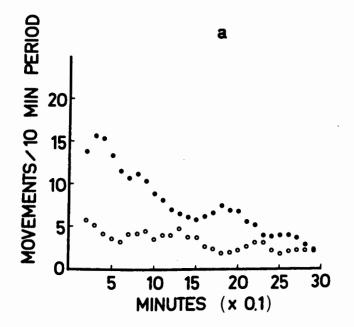
	SIGNIFICA P = 0.05 P = 0.01	SIGNIFICANT DIFFERENCES P = 0.05 ** P = 0.01 ***		not	ITY BY HOUR significant	
VARIABLES	lst Hour	2nd Hour	3rd Hour	4th Hour	5th Hour	đf
i. Food and Water:a. in control areab. in both areasc. in experimental aread. in neither area	_ * * * * * *	* * * * * * * * *	~ * ~ * * *	~ * * ~ * * *	_ * * * * * * * * *	47 101 65 47
<pre>ii. Food and Water Deprivation Followed by Food and Water: a. in control area b. in experimental area c. in neither area</pre>	* * * * * * *	* * * * * * * *	* * * *	* * * * *	\ \\	7 7 7 7
iii. First Opportunity to Explore Experimental Area With Food and Water: a. in both areas b. in experimental area	* *	<u>*</u> **		* \	\\	2 to 2
iv. First Opportunity to Explore Experimental Area After Food and Water Deprivation a. in experimental area	* * *	. /		* *		53
P Values calculated with one-way analysis	of	variance.	F distribution	ı	from Dixon and	63

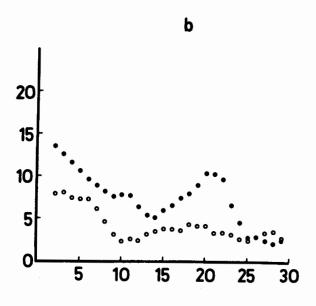
P values calculated with one-way analysis of Massey, 1969, p. 470.

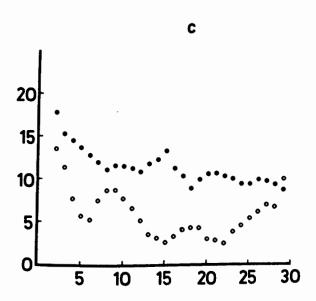
63.

Movements into and out of the experimental area by mice familiar with the experimental area but deprived of food and water for 24 hrs. prior to the experiment.

- a. food and water were placed in the control area
- b. food and water were placed in the experimental area
- c. food and water were not given.







of all in iic where no food was provided.

Ultrasound had significant repelling effects in this series although absolute levels of activity remained higher than in the first series with the whistle operating.

In the third series, mice without previous opportunity to explore the experimental area showed high levels of activity for the first two hours both with the whistle operating and off (Figs. 13 a,b). These initially high levels fell off less rapidly when food and water were presented in the experimental area (iiib), than in iiia where food and water were in both areas. After the third hour, levels in both iiia and b were comparable to those in iiic.

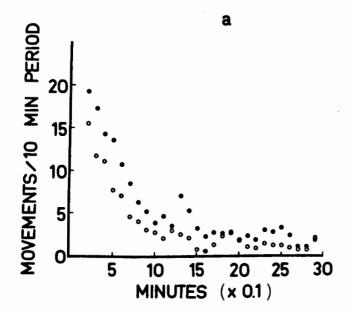
Ultrasound had little repelling effect in this series, with significant reductions in activity in only two of the five hours.

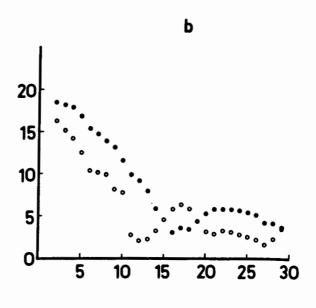
In Experiment 4, mice were deprived of food and water and were restricted to the control area during the 24 hour acclimation period. Food and water were presented in the experimental area at the start of the experiment. With the whistle off initial levels of activity (Fig. 13c) were highest of all four experiments. After two hours, activity was reduced to levels similar to those in iic (Fig. 12c), where mice were without food and water. Ultrasound appeared to have little effect in reducing activity.

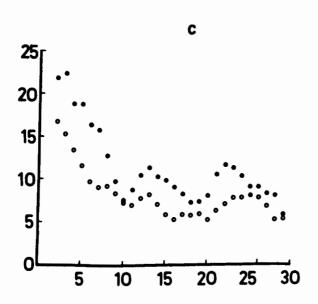
One group of six mice were exposed to 130 db SPL of 19 - 20 kHz ultrasound for three hours. While ether-anaesthetized, their tympani were examined with the otoscope. In all cases, the

Movements into and out of the experimental area by mice unfamiliar with the experimental area.

- a. food and water were placed in both experimental and control areas
- b. food and water were placed in the experimental area
- c. mice were denied food and water for 24 hours prior to the start of the experiment. Food and water were placed in the experimental area.







tympani were ruptured (Fig. 14a). After five to six weeks healing had occurred, but even after a further four weeks only partial hearing had been recovered. Hearing was qualitatively tested using a clicker described in Experiment 4. Decisions on hearing acuity were made from observing Preyer's reflex (flattening of pinnae), or movement of vibrissae, or leaping up followed by crouching.

After as little as one hour exposure to 120 db of ultrasound, damage to tympani occurred in the form of inflammation and local haematomas often followed by thickening and clouding of the membrane within a week (Fig. 14b). Recovery of hearing seemed complete within four to eight weeks.

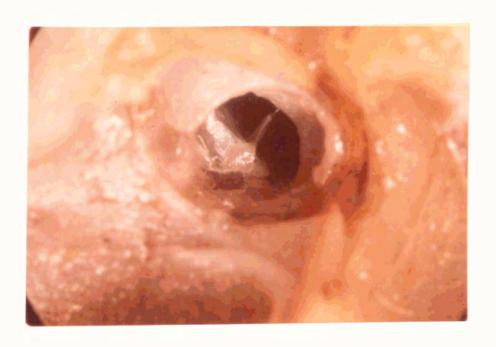
Loss of hearing occurred to animals used in all experiments, but tended to be greatest to those used in the last three series, especially iiia, iiib, and iv. Aggression, measured by observed fighting and bites was much greater in groups of males after being partially or completely deafened. Mortality was higher in these groups for several weeks after exposure to ultrasound.

Macrophotographs of tympani of \underline{M} . $\underline{musculus}$ damaged through exposure to high intensity ultrasound.

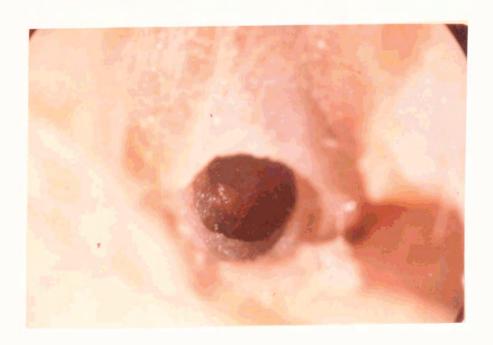
- A: Left tympanum is ruptured. The malleus is covered with the remaining shreds.
- B: The left tympanum of this individual is intact but the middle ear is filled with fluid.

The actual diameter of the auditory canals is 2 - 3 mm.

Α



В



DISCUSSION

The house mouse, unlike the brown rat, tends to explore immediately any changes in his environment (Crowcroft 1966). Mice, even with an excess of food, will browse and sample from many locations rather than develop restricted feeding patterns. However, behaviour is regularized into periods of activity and inactivity ranging from 3/4 hour to 1 1/2 hours.

Fed mice, familiar with their environment and with established sources of food and water were the least active of all in this study. High intensity ultrasound had the strongest repellent effect on these animals in the 5 hour experiments.

Brown (1961) found that rats deprived of food or water run faster to avoid painful or stress-provoking stimuli. Even the amplitude of the unconditioned leap and crouch response to a loud noise is greater. The results of this study do not indicate that food deprivation causes similar changes in the behaviour of Mus musculus. Ultrasound did not appear to repel unfed mice in iic (deprived 24 hours) to a greater degree than it did to either fed mice in ib or unfed mice in id (not deprived of food during the acclimation period) based on comparisons in Table 6.

Commensal rodents, like many other opportunistic species, seek "information" by continuous exploration of new or changing environments. In general, there would be advantages in exploring at least some mild stress-provoking situations. This explora-

tory behaviour must be dependent on the internal state of the animal as much as the stimulating factors. The external situation's effectiveness in evoking exploratory behaviour depends on its relation to the familiar as established by experience. Furthermore, the internal state must influence whether a given degree of discrepancy elicits evasion or exploration and the intensity of either. Changes to the internal state from competing stimuli (e.g. presence of a conspecific) may conflict with exploratory behaviour.

Commensal rodents presented with a novel situation or environment display approach-avoidance behaviour characterized by ever longer forays from the familiar followed by retreats. The initially high levels of activity in iiia and iiib (fed, first opportunity to explore) represent this type of exploratory behaviour with the high number of movements into and out of the experimental area indicating advances and retreats. The presence of food in the experimental area in iiib may account for the maintenance of high levels for a longer period. Crowcroft (1963) found one mouse would explore a 400 ft enclosure for an hour, followed by an equal period of rest. It would appear from the results of iiia and iiib that the temporal components of exploratory behaviour are of more consequence than the spatial.

The minimal repellent effects of ultrasound under these conditions in contrast with experiments i and ii indicate the strength of the urge to explore. The resulting damage to tympani from the ultrasound must have been associated with very unfavour-

able physiological effects. Hebb (1955), suggested that organisms have an optimum level of arousal that is influenced by sensory input. Low levels of input would favour responses that produce increased stimulation and hence greater arousal. Very high or intense sensory bombardment producing high arousal should serve to inhibit the behaviour that leads to this intense stimulation.

If the mice in experiments iii and iv displayed behaviour associated with exploration, the results do not support Hebb's hypothesis. Ultrasound significantly reduced levels of activity in the second hour in iiib (Table 6), but not in iiia. This reduced activity may be a result of reduction in feeding in the second hour in iiib. Levels of activity in the ultrasound were similar in both experiments iiia,b (Figs. 13 a,b), but were higher in iv (Fig. 13c). Ultrasound evidently inhibits feeding more than exploring. When deprived of food and water and presented with a first time opportunity to explore as in iv, the level of activity was higher than in any other experiment (Fig. 13 c) and ultrasound repels significantly for only the first hour. However, levels of activity during the first hour with the whistle operating are not reduced below those levels in iiia and iiib with the whistle silent. The essential difference between iiib and iv was food deprivation. The greater activity in iiib (with the whistle off) which the ultrasound inhibits may be the result of feeding.

The reduced repellent effect of high intensity ultrasound

is most noticeable under conditions where exposure to the ultrasound is high in the first hours. It is possible that when an animal perceives the sound as loud, it is repelled, but if in the sound field sufficiently long, the animal habituates, is deafened, or both. Certainly hearing impairment was greatest for mice used in experiments iii and iv.

Subordinate mice in fighting bouts inhibit further agression from dominant animals by loud squeaking (Eibl-Eibesfeldt 1950, Ralls 1967). The observed fighting among post-experimental males, resulting in the death of some animals, may be due in part to hearing loss. Deafening has also been shown to be involved in the loss of pup retrieval behaviour in response to auditory behaviour (See Experiment 1). Casual observation indicated lower survival rates of litters born to deafened females. The importance of auditory information to a colony of free living wild mice is yet to be determined.

Because of the grainy nature of the films used to record observations, identification of individual mice was not possible, and the social status of animals in the sound field was not known. Thiessen (1966) reports that low status animals tend to be more active and likely to venture into new areas. Different tolerances from high intensity ultrasound may exist for high and low status animals. The effectiveness of the whistle in protecting a new environment may be related not only to the amount of food and cover, but also to the social status of the immigrants. More work is necessary before these questions can be answered.

In management situations, where the home range of individual animals would be 100 - 200 times larger than in this study, exploratory behaviour may be modified, and the repelling effect of ultrasound possibly greater. The behaviour of mice deprived of food and water may be similar to that observed in the laboratory. Certainly the smooth glass walls of the experimental apparatus resulted in greater reflection of ultrasound and possible difficulty for animals in localizing the sound source. This confusion could have led to longer exposures to the ultrasound than would occur in a field situation.

The whistle was most effective when the probability of deafening or habituating to the ultrasound were low, i.e. when animals were exposed to ultrasound for brief periods. Where alternative sources of food are available, such as bait stations in sound free areas, mice in established colonies should be susceptible to management.

CHAPTER II

Field Tests of an Ultrasonic Whistle

INTRODUCTION

The environment of free-living animals, in comparison to their laboratory counterparts, must be considered as being richer in terms of amount and variety of stimuli impinging on individuals. Responses from animals in a laboratory setting may be of a different nature or intensity from those of their wild counterparts. Further, responses to a uniform stimulus presented in different natural settings may vary in ways related to the intrinsic difference between the environments.

Marsh et al. (1962) reported the failure of ultrasound at 15 - 16 kHz and 90 db to disperse colonies infesting grain elevators. Kelih (1963) reduced activity in the sonic field of an electronic device (18 kHz and 100 db at 1 ft.) in grain elevators but could not disperse the colony. Burger (1967) reported only mild aversion towards an air driven whistle (18 kHz and 100 db at 1 ft.), again in grain elevators. These three studies employed relatively low frequency and low intensity devices in environments with a high ambient noise level.

It would be expected that both the number and intensity of responses to stimuli that elicit escape should be related to predation pressure or stability of the habitat. The repellent effect of high intensity ultrasound should be greater in both field situations as compared to laboratory studies.

The present study reports on the repellent effects of high intensity ultrasound on two free-living colonies of <u>Rattus</u> norvegicus. In one test situation, changes in feeding and locomotory activity were measured and in the other situation, changes in all activity outside the burrows was indirectly measured.

A number of ultrasonic devices have been produced as rodent repellents, but little information exists on possible effects on non-target organisms. Effects of high intensity ultrasound on the growth and general behaviour of domestic chickens and a domestic pig are reported here.

1. Repellent Effects of High Intensity Ultrasound on Rats in a Warehouse

Objective

The purpose of the study was to measure changes in activity and feeding in response to ultrasound of a colony of wild \underline{R} . norvegicus feeding, but not nesting, in a small warehouse.

Test Site and Methods:

A small warehouse, actually the lower floor of a corner grocery store, served as the first test site. A small colony of R. norvegicus, nesting outside the building in nearby second growth and debris, had access to the building through at least two openings in the exterior walls. Rats entered the lower floor after dark in the evening and fed on food stuffs stored on islands of shelving in the centre of the one large room 9.5 m x 10.5 m.

The size and movements of the colony were measured by counting droppings and observing tracks across powder spread on the floor. A qualitative measure of activity was achieved by assigning values to the density of tracks in the powder laid down daily. These categories were:

- * single set of tracks across powder
- ** several sets of distinct tracks
- *** tracks over others
- **** powder completely swept away.

This method allowed a direct comparison between movements of the original and residual population, a more important measure than an estimate of absolute density (Chitty and Shorten 1946).

After one week of monitoring activity, three ultrasonic whistles (described in General Methods) were placed in the room (Fig. 15) so as to direct a sound field around the central islands of shelves and the back wall, an area covered with debris. Sound levels within two meters of the islands did not fall below 120 db and did not fall below 95 db anywhere in the room. With the whistles silent, ambient levels varied between 75 and 85 db. For four weeks, including periods when the whistles were both operating and silent, activity of the rats was monitored daily in the five areas indicated in the floor plan.

Week 1 - Whistle off
Week 2 - Whistle on 8 pm to 8 am
Week 3 - Days 1 - 3: Whistle off
Days 4 - 7: Whistle on 8 pm to 8 am
Weeks 4,5 - Days 1 - 3: Whistle on 8 pm to 8 am
Days 4 - 7: Whistle off.

2. Repellent Effects of High Intensity Ultrasound on Rats in a Chicken Barn

Objective

The purpose of this study was to measure changes in activity in response to ultrasound of a colony of wild \underline{R} . norvegicus feeding and nesting in a large chicken barn.

Test Site and Methods:

A small colony of \underline{R} . norvegicus burrowing, nesting and feeding in the test area served as subjects to measure the effectiveness of high intensity ultrasound in extirpating rodents.

Floor plan of basement of Highway Market

Area 1 - Food storage shelves

Area 2 - Floor beneath food storage shelves

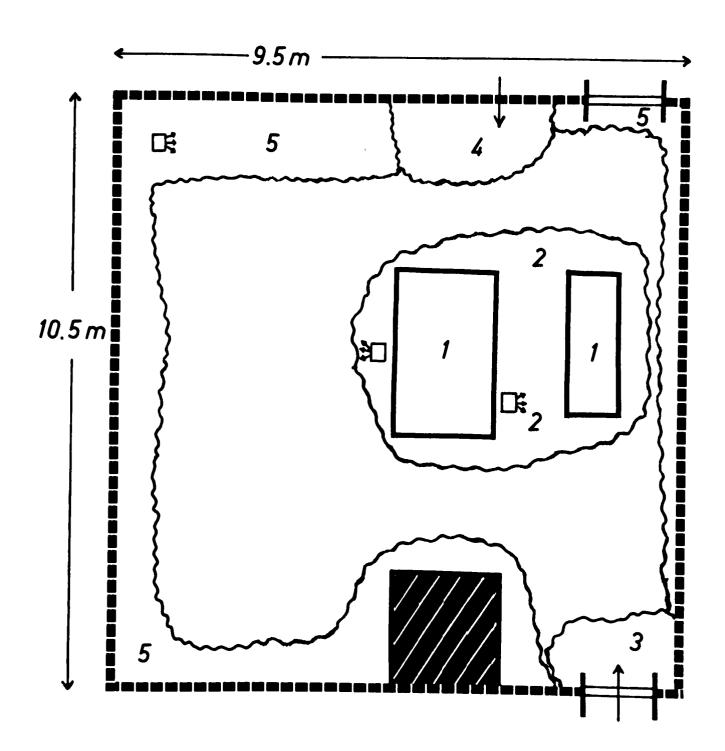
Area 3 - Entrance to crawlspace

Area 4 - Rear entrance (hole in wall)

Area 5 - Periphery of room

= entrance for rats

= whistle



This colony was living in the concrete lined pit beneath the lathing floor of a chicken barn 7.47 m x 60.03 m (Fig. 16). The pit was 1.5 m deep, and was filled with wood shavings and droppings to a depth of 0.5 m. The steady buildup of chicken droppings and rich supply of spilled chicken feed, broken eggs and droppings themselves precluded an accurate measurement of population size and movement employing conventional techniques such as tracking powder, census baiting and trapping. However, with the continual rain of droppings from 5,000 hens, only active burrows remained open for more than a day. A daily count of open burrow entrances thus offered a relative measure of activity, and this technique was employed throughout the study. Tracking powder was used to detect movements of rats from the barn at the single point accessible to them.

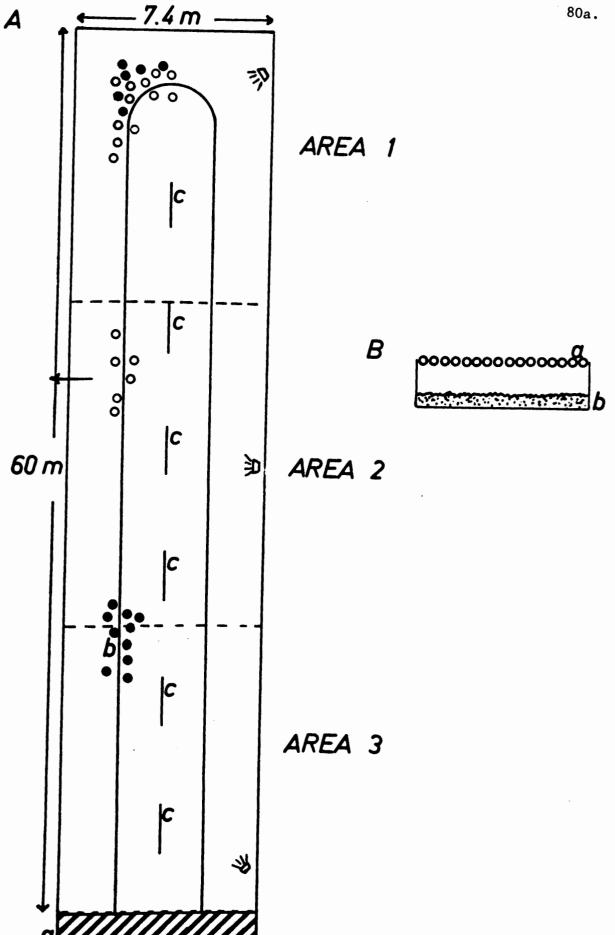
After one week of observation, three ultrasonic whistles (described in General Methods) were placed beneath the lathing floor of the barn so as to direct a diffused unobstructed sound field over the surface of the entire pit, except as otherwise indicated. For four weeks, including periods when some or all of the whistles were operating or silent, the number of open burrow entrances in all areas and signs of rats leaving the barn were monitored daily. Five evening observations were made in Weeks 1, 2 and 3 with an infra-red Snooperscope to observe rat activity above the lathing.

- A. Floor plan of hen house
 - a. Feed preparation area. Rats did not have access to this area.
 - b. Automatic U-shaped chain driven feeder ran the full length of barn and was suspended from the ceiling.
 - c. Six automatic watering troughs

= Exit for rats

= Whistle

- B. Cross section of pit
 - a. Lathing floor
 - b. Wood shavings and chicken droppings.
 - = active burrow entrances at the start of the experiment
 - active burrow entrances at the end of the experiment.



Schedule of Whistle Operation.

Week	Area 1	Area 2	Area 3
1 2 3 4 5 (Day 1 - 4) 5 (Day 5 - 7)	off on off on on off	off on on off on	off on (8 am - 8 pm) on off on off

3. Effects of High Intensity Ultrasound on Chick Growth and Behaviour

Objective

The purpose of this study was to measure the effect of ultrasound on the growth and development of domestic chicks.

Methods and Materials:

Six, day-old Paymaster rooster chicks, <u>Gallus domesticus</u>, served as subjects in this experiment. Experimental and control groups of three were raised separately in 3 m x 3 m rooms maintained at 20° C + 2° and illuminated from 0800 hours to 2000 hours. The experimental group of rooster chicks were exposed to 20 kHz of ultrasound at 120 db SPL at 1 m for a period of five weeks from 2000 hours to 0800 hours. The control group was exposed to noise from the compressor of 82 db, 4 db over the ambient level, for a similar period. Experimental and control regimes were then reversed for a further two week period.

4. Effects of High Intensity Ultrasound on the Growth of a Pig

Objective

The effects of high intensity ultrasound on the growth of a domestic pig were measured in this study.

Methods and Materials:

The rate of growth for a newly weaned 2 month old Langcastershire piglet, Sus scrufa, was established in a three week period. The animal was housed in a 5m x 3 m room with natural lighting. The piglet was then exposed to ultrasound of 20 kHz at 130 db at 3 ft from 2000 hours to 0800 hours until the experiment was terminated. Ambient sound levels during the day were approximately 72 db.

Results

1.

From surveying the amount of food taken, the density of tracks in the powder, and the number of burrows near the building, the colony was estimated to consist of between eight and twelve adult rats. Activity was greatest after 2300 hours (the store closing hour), and while not restricted to any one area of the room (Fig. 17), was greatest in the areas near the entrances and the food storage shelves. As is common with rats, movement was greatest near vertical surfaces and sources of cover. From the beginning of the test period, feeding, which generally accompanied activity in Area 1, was inhibited for two weeks, including a three day period when the whistle was switched off. Rats were active near the entrances to the room after only a week of the whistle's operation. Activity gradually increased to that level observed prior to the onset of the whistle's operation.

2.

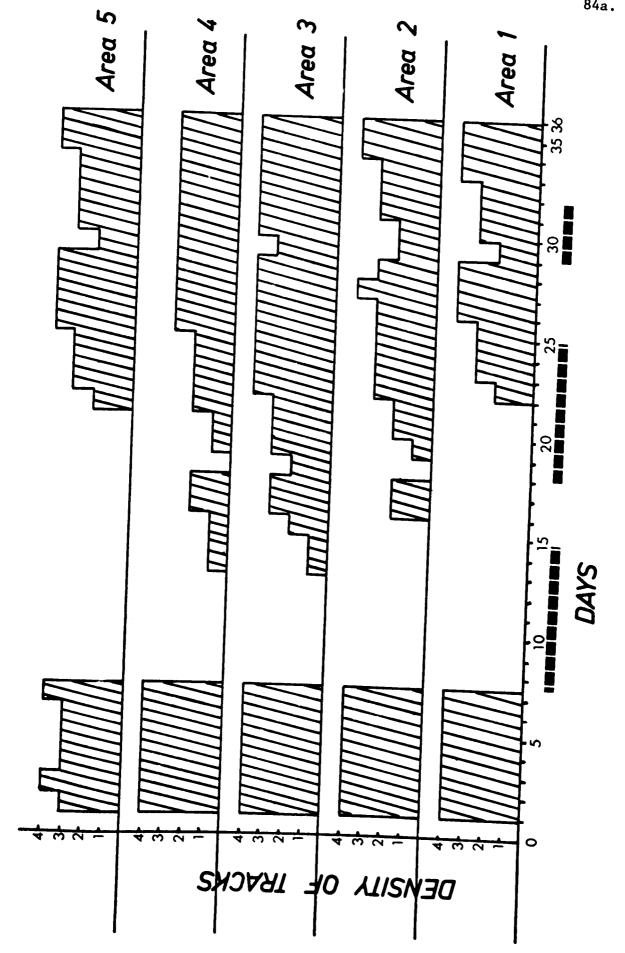
The colony was estimated, on the basis of the number of burrow entrances (Calhoun 1962), to consist of ten to twelve weaned rats. Activity was most probably restricted to the evening hours, and from Snooperscope observations, was restricted to the pit below the flooring, although several broken lathes could have allowed access to the main floor. Food requirements could be met without leaving the pit. The droppings and broken

Figure 17

Effects of ultrasound on activity of the rats in a warehouse as measured by the density of tracks in the following areas:

- 1. Food storage shelves
- 2. Floor beneath food storage shelves
- 3. Entrance to crawlspace
- 4. Rear entrance (hole in wall)
- 5. Periphery of room.

= periods when the ultrasonic whistle was operating



eggs may have supplied sufficient water. Rats were not observed drinking from the water troughs above the floor.

At the beginning of the test period, one of the two burrow systems was centered in a small area at the top of the barn in Area 1. The second burrow system was more diffuse, and spread in a line under the automatic feeder in Area 2. From Table 7, it is clear that overall activity, as measured by the number of open burrows, was greatly reduced in the first week of the whistle's operation, and activity in Area 2 was reduced to the same level for a second week. The rats in this burrow system probably dispersed into Area 3 in the third week of the whistle's operation. From the tracking powder there was no evidence that any animal left the barn.

3.

There was no difference in the rate of growth of chicks in the two groups (Fig. 18). Social behaviour was not obviously different between the two groups, either in the first or in the second phase of the experiment. The reduction in the rate of growth in both groups results from a change in feed.

4.

Although high intensity ultrasound appeared not to affect the weight gain of the pig (Fig. 19), the whistle was switched off after one week. The animal showed extreme irritability to noises, approach by experimenters, and sudden movements. Also.

Effects of Ultrasound on the Number of Active Burrows in the Chicken Barn

		ACTIV	E BURROWS		
	Day	Area #1	Area #2	Area #3	TOTAL
Jan. 11 1972	12345678901234567890123456 12121111111122223456	12 14 14 14 11 12 23 23 22 1 48 99 11 10 12 23 4	668987801202221001223467	000000 x 0	18 18 22 23 218 20 19 23 43 443 69 99 12 12 13 15 15
	26 27 28 29	$\begin{array}{ccc} x & \overline{x} \\ 4 & 3 \end{array}$	x x x 7 5.4	$\begin{array}{ccc} x & \overline{x} \\ x & \overline{x} \\ 5 & 3. \end{array}$	$\begin{array}{ccc} x & \overline{x} \\ x & \overline{x} \\ 16 & 8.1 \end{array}$
	23 24 25 27 29 29 31 33 33 35 35	1 4 3 x 3 2.8 6 8 x 7	3 4 2 4 3.3 4 7 5 4.3	2 2 2 2 2 0 3 4 3 3 3	$ \begin{array}{cccc} 6 & & & \\ 10 & & \overline{x} \\ 7 & & \overline{x} \\ 9 & & 8. \\ 13 & & \overline{x} \\ 16 & & \overline{x} \\ 15 & & 14.7 \end{array} $
Feb. 21	35	7 7	4 x 5 4.3	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$ \begin{array}{cccc} 16 & \overline{x} \\ 15 & 14.7 \end{array} $

x - NO DATA

Note: the burrows in Area 2 from day 23 on were within a a meter of Area 3

= Ultrasound on at this time in this area

Figure 18

Growth rate of rooster chicks in the ultrasound field. Three chicks were exposed to 19 - 20 kHz at 120 db together with the noise of the compressor driving the whistle; three chicks were exposed to low frequency noise (82 db) from the compressor alone.

- ooo chicks in ultrasound
 - - feed was changed for both groups
 - control and experimental regimes were reversed, ie.

 chicks previously in ultrasound were exposed to

 compressor noise and chicks in compressor noise were

 exposed to ultrasound.

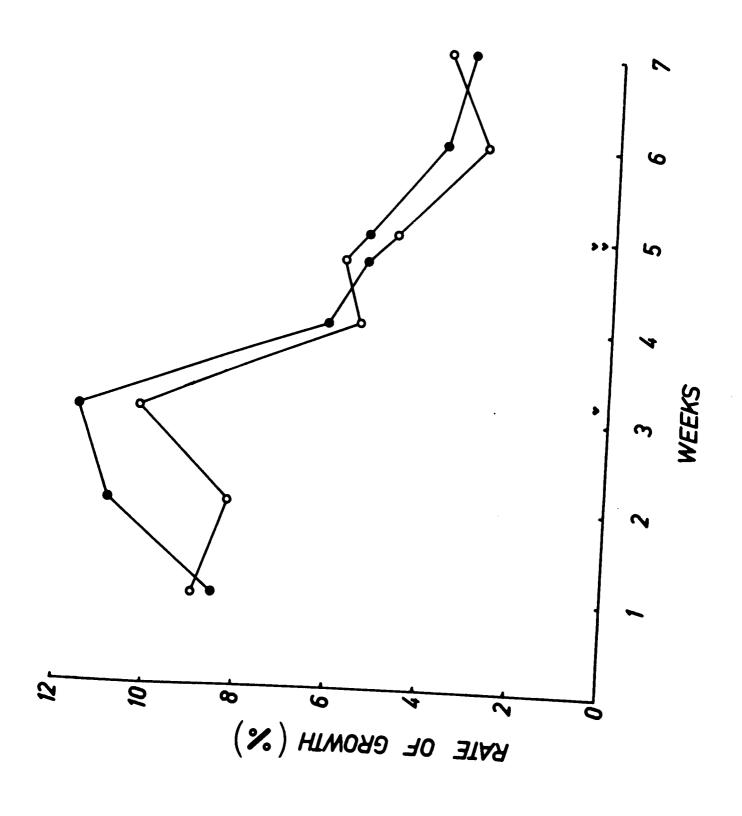
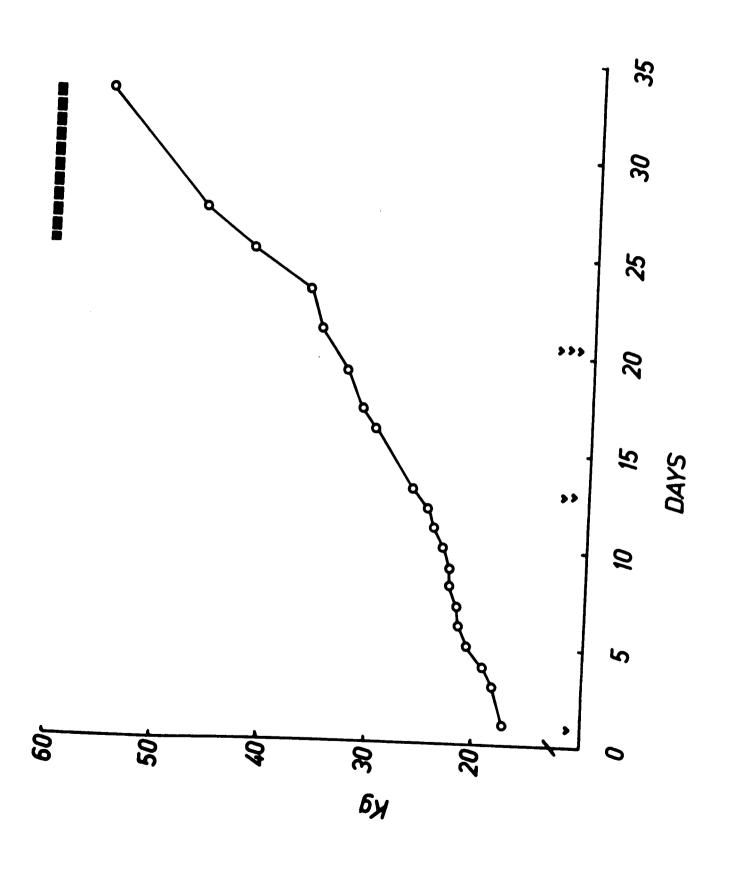


Figure 19

Growth of a domestic piglet in an ultrasound field. From the 23rd to the 33rd day of the study the piglet was exposed to ultrasound of 19 - 20 kHz at 130 db between 2000 hours to 0800 hours.

- → piglet was 2 months old at the beginning of the study
- piglet was dewormed
- work was done on the pen housing the animal

ultrasound was on



in the last three days of the whistle's operation and for several days following, the pig's food consumption was reduced by a third.

Discussion

The rats were frequenting the warehouse presumably as a result of attraction to the stored foodstuffs whereas the chicken barn constituted a complete home range for the colony living in it. The greater success in restricting activity in the warehouse probably reflects the natural proclivity of rats to maintain a rather small, well-defined home range which they are reluctant to leave. Indeed, Davis et al. (1948), Calhoun (1962) and Emlen et al. (1949) report extremely low levels of migration from areas where the habitats were destroyed. Nass et al. (1971) report similar findings for Rattus rattus during sugar cane harvesting operations.

Although levels of activity in the chicken barn as a result of the whistle's operation were reduced for comparable periods of time, this reduction was not to the same degree as in the warehouse, and probably reflects an intrinsic difference between the two test situations and colonies. In both cases, feeding in the ultrasonic field was restricted, but nesting in the chicken barn was affected little.

In the warehouse, the levels of ultrasound were higher near the food storage shelves than near either of the entrances. These higher levels, together with the open floor space around the shelves, account for activity being restricted to the entrances in the second week of the whistle's operation.

Alternative food sources for the colony were not obvious and hunger may have been a strong factor in the habituation of the

animals to the sound.

As measured by the number of open burrow entrances in the chicken barn, the whistle had a substantial effect in the first week of operation. This effect slowly lessened over the following two weeks. The establishment of a new burrow system in Area 3 appeared to be a direct result of the whistle's operation. A burrow system is never static, but complete abandonment, as observed in this situation, must be the result of considerable pressure.

Ultrasound, independent of other control measures, offers excellent short term control, but its effects wane as a function of both time and type of behaviour involved, e.g. feeding vs. nesting. The repellent effects are not sufficient to permanently disperse an established colony. It is of small value as a long term single form of control. The potential of ultrasound in protecting uninhabited environments from infestation remains to be explored. Also, ultrasound could be of considerable value in integrated control programs, especially in situations where poison or trapping alone are not feasible, e.g. stored food infestations. In conjunction with a prebaiting program in a convenient, sound-free area, ultrasound could be a valuable control measure.

The hearing of birds tends to be attuned to a rather narrow range of frequencies in the middle of the human audible range (Schwartzkopff, 1955). The hen's hearing is most sensitive to sounds in the range of 3 kHz, which corresponds well with the

chick's cries of anguish (sic.) (Busnel 1963). The upper limits of the chicken's hearing does not exceed 20 kHz and their inability to hear the whistle would appear to account for its lack of effect on their growth and development.

Exposure over several weeks to intermittent aircraft noise of frequencies ranging from 200 to 4500 Hz at 104 to 120 db had no effect on rate of gain of domestic pigs, <u>Sus scrufa</u>, although the animals never completely habituated and showed some initial alarm at the onset of the noise (Bond 1970). Continuous white noise (composed of a broad spectrum of frequencies) at 93 db over several days caused aldosteronism and severe retention of water and sodium in young pigs (Bugard <u>et al.</u> 1960). The grunting sounds of the domestic pig are in the range of 250 Hz to 320 Hz and their screams of distress and pain do not exceed 1.5 kHz (Busnel 1963), but from the results of the present study, the pig's hearing must exceed 20 kHz. Sensitivity to ultrasound would preclude its use in control programs near these animals.

GENERAL CONCLUSIONS

Juvenile rats emit ultrasonic vocalizations in response to intense stimulation. These cries are similar to cries that have previously been interpreted as inhibiting aggression or perhaps contact. The precise communicative value remains to be found. Possibly these or similar calls could be exploited in control programs. I have shown the communicative value of the ultrasonic cries of isolated mouse pups. Recordings of these cries will attract lactating females over short distances. Deafened mice do not leave the nest to retrieve pups and are probably more aggressive towards their young as well as conspecifics. Deafening should be investigated as a control technique.

High intensity ultrasound, such as that produced by the whistle used in this study, would not be practical as a deafening agent for rats as relatively long exposure times are necessary. The ultrasound may interfere with perception of intra-specific auditory signals and certaintly must reduce the rat's echolocating abilities.

The repellent effects of high intensity ultrasound are moderate under laboratory conditions. Repulsion is greater when rats or mice are given alternative sources of food and water in sound-free areas. Mice are more active when deprived of food and water or when given an opportunity to explore a novel area. Although the repellent effects of the whistle were slight in these situations, hearing loss was involved and the animals may not have perceived the ultrasound as being stressful.

Free-living animals were repelled for longer periods than rats in the laboratory. However, as with laboratory findings, degree of repulsion was related to internal states of the animals. Feeding behaviour was more strongly affected than nesting. The rats eventually responded less to the ultrasound in both field tests. The loss of response in laboratory or field situations could be produced by a variety of learning processes, the simplest of these being habituation or loss of response to stimuli that are not associated with "reward or punishment" (p.28). Conditioning may also occur, and classically this would involve the association of the noxious stimulus with other "harmless" stimuli such as the appearance of the experimental cage or equipment. (type I conditioning, Thorpe 1963 p. 79) In the absence of specific experiments to discriminate between these two learning processes, I have used the term habituation.

Habituation occurs less rapidly when a sound stimulus is associated with physiological damage (Galambos 1956). However, after chronic exposure to high intensity ultrasound, both perception of the sound and the possibility that further physiological damage could occur must be reduced. The repellent effects of the sound could be made more persistent through simultaneous stimulation of another sensory modality (e.g. vision). Eradication, rather than repulsion, would be possible with a program integrating ultrasound with pre-baiting and poisonbaiting in sound-free areas. This technique should be of value in situations such as stored food infestations when poisoning and trapping are either impossible or undesirable.

APPENDIX I: 8

	Total Hours Observed (N)									
	Ī	0 7	42	₽#	76	85	57	52	29	37
	Visits per Hour for Neither	2.3 ± 0.6	1.4 ± 0.4	1.3 ± 0.4	1.9 ± 0.3	1.0 ± 0.2	1.3 ± 0.3	1.0 ± 0.2	0.8 ± 0.2	1.1 ± 0.3
	Visits per Hour for Water	0.6 ± 0.1	0.8 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.8 ± 0.2
- STANDARD ERRORS	Visits per Hour for Hood	0.9 + 0.3	0.4 ± 0.2	0.5 ± 0.2	0.1 ± 0.1	0.2 ± 0.1	0.4 + 0.1	0.7 ± 0.2	0.4 ± 0.1	0.9 + 0.4
MEANS	Average Amount of Time per Visit in Sound Field (sec.)	4.3 ± 0.6	7.3 ± 1.5	5.1 ± 0.6	2.1 ± 0.3	3.6 ± 0.4	4.2 ± 0.5	4.3 ± 0.6	5.3 ± 0.6	5.4 ± 0.8
BASIC STATISTICS FROM EXPERIMENT 4:	Average Amount of Time per Hour in Sound Field (sec.)	22.9 ± 4.8	24.4 ± 5.7	14.2 ± 2.6	6.0 ± 8.8	9.5 ± 1.2	12.0 ± 2.0	13.0 ± 2.4	12.7 ± 1.7	18.1 ± 3.5
STATISTICS FRO	Length of Visits to Experimental Cage (sec.)	44.1 ± 12.1	48.6 ± 12.8	129.8 ± 74.8	33.7 ± 12.5	112.3 ± 36.0	112.6 ± 51.5	202.8 ± 66.8	288.8 ± 97.7	66.7 ± 18.1
BASIC	Visits per Hour to Experimental n Area	3.7 ± 0.7	2.6 ± 0.5	2.1 ± 0.5	2.1 ± 0.3	1.8 ± 0.3	2.3 + 0.4	2.2 + 0.4	1.8 ± 0.3	2.8 ± 0.6
	Total Time in Experimental Cage % of Observation Period	5.6 ± 1.8	3.5 + 0.8	5.4 + 2.1	2.4 + 0.8	5.0 ± 1.2	5.1 ± 1.5	7.2 ± 1.9	10.1 ± 2.4	6.0 + 4.4
	Day	1	a	W	#	5	9	7	ω	0

APPENDIX I: b

BASIC STATISTICS FROM EXPERIMENT 5: MEANS ± STANDARD ERROR

1 5.5 ± 1.8 3.7 ± 0.7 62.8 ± 18.8 17.0 ± 4.4 4.3 ± 0.6 0.2 ± 0.1 0.2 ± 0.1 2.0 ± 0.5 43 2 4.5 ± 1.3 2.6 ± 0.5 52.4 ± 20.3 21.5 ± 5.0 4.2 ± 0.6 0.5 ± 0.3 0.6 ± 0.1 2.4 ± 0.5 39 3 3.1 ± 0.7 2.1 ± 0.5 57.1 ± 15.0 13.5 ± 2.4 4.5 ± 0.6 0.4 ± 0.2 0.2 ± 0.1 1.5 ± 0.3 61 4 1.6 ± 0.5 2.1 ± 0.3 14.0 ± 3.3 8.8 ± 1.7 2.9 ± 0.5 0.0 ± 0.0 0.1 ± 0.0 2.1 ± 0.3 72 5 2.2 ± 0.6 1.8 ± 0.3 23.4 ± 4.7 13.5 ± 2.5 4.7 ± 0.8 0.2 ± 0.1 0.1 ± 0.1 1.6 ± 0.3 57 6 3.6 ± 1.1 2.3 ± 0.4 56.7 ± 15.0 10.5 ± 1.9 4.0 ± 0.5 0.1 ± 0.0 0.1 ± 0.0 1.4 ± 0.2 76 7 5.2 ± 1.5 2.2 ± 0.4 86.5 ± 40.6 13.5 ± 2.6 3.5 ± 0.5 0.1 ± 0.0 0.2 ± 0.1 1.5 ± 0.3 60 8 8.1 ± 2.3 1.8 ± 0.3 141.8 ± 54.1 15.0 ± 3.4 4.2 ± 0.6 0.1 ± 0.0 0.2 ± 0.1 1.5 ± 0.4 67 9 5.4 ± 0.9 2.8 ± 0.6 46.3 ± 9.4 13.1 ± 2.8 4.6 ± 0.7 0.1 ± 0.1 0.4 ± 0.1 1.3 ± 0.3 51	Day	Total Time in Experimental Cage % of Observation	Visits per Hour to Experimental n Area	Length of Visits to Experimental Area (sec.)	Average Amount of Time per Hour in Sound Field (sec.)	Average Amount of Time per Visit in Sound Field (sec.)	Visits per Hour for Food	Visits per Hour for Water	Visits per Hour for Neither	Total Hours Observed (N)
4.5 ± 1.3 2.6 ± 0.5 52.4 ± 20.3 21.5 ± 5.0 4.2 ± 0.6 0.5 ± 0.3 0.6 ± 0.1 2.4 ± 0.5 0.5 1	٦	1+1	3.7 ± 0.7	+1	17.0 ‡	+1	+1	+1	+1	43
3.1 ± 0.7 2.1 ± 0.5 57.1 ± 15.0 13.5 ± 2.4 4.5 ± 0.6 0.4 ± 0.2 0.2 ± 0.1 1.5 ± 0.3 14.0 ± 3.3 8.8 ± 1.7 2.9 ± 0.5 0.0 ± 0.0 0.1 ± 0.0 2.1 ± 0.3 2.2 ± 0.6 1.8 ± 0.3 23.4 ± 4.7 13.5 ± 2.5 4.7 ± 0.8 0.2 ± 0.1 0.1 ± 0.1 1.6 ± 0.3 3.6 ± 1.1 2.3 ± 0.4 56.7 ± 15.0 10.5 ± 1.9 4.0 ± 0.5 0.1 ± 0.0 0.1 ± 0.0 1.4 ± 0.2 5.2 ± 1.5 2.2 ± 0.4 86.5 ± 40.6 13.5 ± 2.6 3.5 ± 0.5 0.3 ± 0.1 0.3 ± 0.1 1.5 ± 0.3 8.1 ± 2.3 1.8 ± 0.3 141.8 ± 54.1 15.0 ± 3.4 4.2 ± 0.6 0.1 ± 0.0 0.2 ± 0.1 1.6 ± 0.4 3.4 ± 0.9 2.8 ± 0.6 46.3 ± 9.4 13.1 ± 2.8 4.6 ± 0.7 0.1 ± 0.1 0.4 ± 0.1 1.3 ± 0.3	0	+ 1	++	+1	21.5 ±	+ı ∾	رح 1+	+1	; +1	39
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3.6 ± 1.1 2.3 ± 0.4 56.7 ± 15.0 10.5 ± 1.9 4.0 ± 0.5 0.1 ± 0.0 0.1 ± 0.0 1.4 ± 0.2 5.2 ± 1.5 2.2 ± 0.4 86.5 ± 40.6 13.5 ± 2.6 3.5 ± 0.5 0.3 ± 0.1 0.3 ± 0.1 1.5 ± 0.3 8.1 ± 2.3 1.8 ± 0.3 141.8 ± 54.1 15.0 ± 3.4 ± 0.5 0.1 ± 0.0 0.2 ± 0.1 1.6 ± 0.4 5.4 ± 0.9 2.8 ± 0.6 46.3 ± 9.4 13.1 ± 2.8 4.6 ± 0.7 0.1 ± 0.1 0.4 ± 0.1 1.3 ± 0.3	5	+1	+1	+1	++ 2.	; +1	; +ı	+1	+1	57
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8.1 ± 2.3 1.8 ± 0.3 141.8 ± 54.1 15.0 ± 3.4 4.2 ± 0.6 0.1 ± 0.0 0.2 ± 0.1 1.6 ± 0.4 5.4 ± 0.9 2.8 ± 0.6 46.3 ± 9.4 13.1 ± 2.8 4.6 ± 0.7 0.1 ± 0.1 0.4 ± 0.1 1.3 ± 0.3	7	+1	+1	-7 +1	ن ۱+	5 ++	+1	+1	+1	90
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	9	3.4 ± 0.9	++	+1	+1	+1	+ 0.1	5.1	1.3 ± 0.3	51

APPENDIX I: c

	Total Hours Observed (N)	64	27	18	94	21	21	12	18	18
MEANS + STANDARD ERROR	Average Amount of Time per Visit in Sound Field (sec.)	6.5 ± 1.1	8.5 ± 2.2	8.3 ± 2.0	6.3 ± 1.4	5.0 ± 1.0	5.1 ± 1.0	10.9 ± 1.8	12.6 ± 5.2	11.0 ± 3.7
FROM EXPERIMENT 6:	Average Amount of Time per Hour in Sound Field (sec.)	18.0 ± 4.0	30.0 ± 7.4	23.7 ± 6.1	16.2 ± 3.9	19.4 ± 5.7	17.8 ± 5.3	40.1 ±10.6	24.4 ± 5.6	23.4 ± 7:3
BASIC STATISTICS	Total Time in Experimental Cage % of Observation Period	8.2 ± 3.2	5.7 ± 2.3	2.9 ± 1.3	2.5 ± 1.3	2.5 ± 1.6	2.0 ± 0.8	4.5 ± 1.4	7.8 ± 5.5	9.1 ± 5.7
	Day	ч	0	8	†	72	9	7	ω	6

APPENDIX II: a

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APPENDIX II: b

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iiib	$\mu = N$ WITH SOUND	$\begin{array}{c} 24411\\ 044\\ 044\\ 044\\ 044\\ 044\\ 044\\ 044\\$
ii	V = V WITHOUT SOUND	######################################
ia	h = N HTIW SOUND	
iiia	μ = N WITHOUT SOUND	
ን ተልዥ	PERIOD	00001000000000000000000000000000000000

AVERAGE MOVEMENTS INTO AND OUT OF EXPERIMENTAL AREA FOR EACH 10 MIN. PERIOD # STANDARD ERRORS

APPENDIX III.

REPELLENT EFFECTS OF HIGH INTENSITY ULTRASOUND ON MOUSE ACTIVITY, SIGNIFICANCE

	WAS DETERMINED	THROUGH A	ONE WAY	ANALYSIS OF	VARIANCE (P	= 0.05)	
		F VALUES b	by hour		/= not	t significant	nt
	VARTABLES	H		2	7		
		veme	movements	movements	movements	movements	degrees of freedom
	FOOD AND WATER: a. in control area b. in both areas c. in exptl. area d. in neither area	41.04 25.97 10.46	11.47 26.90 16.46	31,94	19,67	12.42 11.83 9.55	47 101 655 47
	DEPRIVATION (24 hrs. FOLLOWED BY FOOD AND WATER a. in control area b. in exptl. area c. in neither area	21.05	12.23	5.54 7.30 17.54	4.08 15.51 18.83	\\\	<u> </u>
	OPPORTUNITY TO EX- PLORE EXPIL. AREA WITH FOOD AND WATER a. in both areas b. in exptl. area	5.43	8,16	64.4		. \\	27 27
	OPPORTUNITY TO EX- PLORE EXPIL, AREA AFTER FOOD AND WATER DEPRIVATION (24 hrs.) FOOD AND WATER a. in exptl, area	17.48	,		4.30		53
1							

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