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Female Mouse Odors: Role of Male Learning.

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Female Mouse Odors: Role of Male Learning

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MASTER'S THESIS ABSTRACT

The "pheromone" concept, first introduced in insect communication research in the early 1950's, was adopted into mammalian chemocommunication research of the early 1960's. One line of mammalian chemocommunication research which appeared to fit the criteria for pheromonal communication was the male house mouse (Mus musculus) ultrasonic response to female odors.

Among male mice, 70 kHz ultrasonic vocalizations are emitted mainly during courtship behavior. Since ultrasonic emissions correlate so well with male sexual arousal and are so well-defined, the ultrasonic response has been used as an indicator of male sexual responsiveness, and also as a measure of the sex-signalling value of various female odoriferous stimuli.

In previous mouse chemocommunication research, female-soiled cage shavings, vaginal secretions, female facial substances, and female urine were found to elicit ultrasounds from males. Further, female urine did not elicit ultrasounds from adult males who had received no previous exposure to either adult females or males (i.e. social-experience). In further exploration of the

constraints upon mouse ultrasound elicitation by odoriferous stimuli, a perfume, previously neutral for ultrasound elicitation, acquired ultrasound eliciting ability when appropriately paired with females.

The following two experiments systematically assessed the effect of exposure to adult females and males in the development of the differential response to male and female urine by adult males. More specifically, the following concerns were explored:

1. The possibility that exposure to urine alone (in the absence of a female mouse) would be sufficient for the development of ultrasound elicitation by urine;

2. The number of times a male must be exposed to a female for the development of the ultrasonic response to urine;

3. The ability to experimentally alter the normal signalling value of male and female urine.

In the first experiment, repeated exposure to both males and females caused female urine to acquire ultrasound eliciting ability, confirming earlier work. Ultrasonic response to female urine by the group receiving repeated exposures to both males and females reached its maximum after three 3-minute exposures to both males and females, suggesting that males are biologically predisposed to respond to female urine. The group that received no exposure to males or females while receiving

exposure to male and female urine swabs emitted virtually no ultrasounds to either male or female urine. These findings indicate that exposure to female and male urine alone is not sufficient to cause urine to acquire ultrasound eliciting potency; the female urine must be paired with a female.

The second experiment explored whether the signalling value of male and female urine could be altered by pairing urinary cues with animals of the opposite sex. Further, the time course for this biologically relevant learning was again examined, followed by attempts to observe the learning phenomena of extinction and spontaneous recovery.

Consistent with Experiment 1, ultrasonic response to female urine by both groups during the acquisition phase reached its maximum after two 3-minute exposures to the social-experience animals. During the extinction phase, ultrasonic response to male and female urine alone declined across trials. Spontaneous recovery was not demonstrated.

Pairing male urine with females and female urine with males failed to alter the normal signal value of male and female urine. This failure was probably due to design problems and not a reflection of an inherent inability to alter urine's normal signalling value.

The above experimental findings are consistent with the hypothesis that elicitation of male ultrasounds by

female urinary odors may be an instance of classical conditioning. Some as yet undetermined aspect of the female's phenotype appears to be the unconditioned stimulus, with female urine being the conditioned stimulus.

CHAPTER 1

Introduction

The Pheromone Concept

The "pheromone" concept was first introduced in the late 1950's in insect communication research (Shorey, 1976). Pheromones were originally conceived as being "external hormones," any chemical substance secreted or excreted by one individual which causes a behavioral or physiological change in another individual of the same species (Shorey, 1976; Beauchamp, Doty, Moulton, and Mugford, 1976). Pheromonal communication was pictured in terms of a simple stimulus-response model. For example, male houseflies attempt to copulate with pheromone-treated knots of string. In this example, the behavioral response is stereotyped, and the chemical cue is relatively simple in structure (Shorey, 1976).

Four criteria, some of which have been used by various investigators (Beauchamp et al., 1976), identify a chemical substance as pheromonal: 1. the behavioral or physiological responses in question must be well-defined; 2. the response must be primarily genetically determined; 3. only one or at most a relatively few compounds must be involved in the elicitation of the

response; 4. the communication must be specific to the species under study (Shorey, 1976; Beauchamp et al., 1976). These criteria were of significant heuristic value in insect communication research and indeed many chemical substances fit some of these criteria well.

In the early 1960's pheromones received increasing attention, and the term came into widespread use in both scientific and popular literature (Beauchamp et al., 1976). In the late 1960's, E. O. Wilson introduced the distinction between "releasing" pheromones and "primer" pheromones (Shorey, 1976). Releaser pheromones quickly "release" specific behaviors, while primer pheromones elicit slower reacting neuroendocrine changes (Beauchamp et al., 1976; Shorey, 1976).

Mammalian chemical communication became a popular research topic starting in the mid- and late 1960's at a time when the study of insect pheromones was already a sophisticated field of research (Beauchamp et al., 1976). Because primates, rodents, and other small mammals are richly endowed with odor producing glands, olfaction was suspected of playing an important role in mammalian social communication (Epple, 1974; Stoddart, 1974). Subsequent research demonstrated olfactory communication to be vital in intra-specific social communication (Doty, 1976; Beauchamp et al., 1976).

The simple stimulus-response model used for insect communication was quickly applied to mammalian communication research. It was common in the published literature to see references to "sex," "aggression- promoting," "aggression-inhibiting," "territorial," and "fear" pheromones in rodents. Unfortunately, the picture such terms create is of simple compounds which unequivocally either release or inhibit a stereotyped response (Beauchamp et al., 1976).

Problems with Pheromone Concept

1. Chemical Complexity

As researchers studied the role of olfaction in mammalian chemical communication, they soon discovered that mammalian pheromones appeared to be complex in chemical composition (Epple, 1974; Bronson, 1974; Stoddart, 1974; Beauchamp et al., 1976). Urine, for example, is a basic medium of chemical communication among mammals (Bronson, 1976), and hundreds of substances of varying molecular weights have been identified in the urine of most mammals. Despite concentrated efforts by many investigators, the chemical composition of only six mammalian "pheromone" systems has been purportedly established (Beauchamp et al., 1976).

Because of the apparent chemical complexity of substances involved in mammalian chemical communication,

Beauchamp et al. (1976) suggest that the implied analogy between insect and mammalian systems is inappropriate, and that the analogy between human responses to food odors and mammalian olfactory communication may be better. Food aromas are generally complex with no one component dominating. If mammalian chemical communication is similarly complex, then to designate only effects mediated by a single chemical as pheromonal is to dichotomize falsely. Interestingly, traditional conditioning studies have found that anything that can make the conditioned stimulus more discriminable facilitates the conditioning process (Beauchamp et al., 1976). Therefore, a mixture of odorants might be more effective in intra-specific chemical communication than single compounds. Mixtures can be varied along a variety of dimensions while single compounds are restricted to the intensity dimension. This hypothesis is consistent with the observation that many mammals are able to identify individuals of their own species by odor cues, suggesting that mammals are able to interpret complex chemical signals. For example, unfamiliar non-castrate male mouse odor blocks the pregnancy of a recently inseminated female mouse, whereas a familiar mouse's odor does not, suggestive of complex chemical communication (Beauchamp et al., 1976).

The chemical complexity of mammalian excretions and secretions does not prove that chemical communication is

complex in chemical structure. However, more study needs to be done before generalizations concerning such complexity can be made. It is premature to anticipate now that only one or at most a few compounds will form the basis for chemical communication in most mammalian species (Beauchamp et al., 1976).

2. Plasticity of Response

In mammalian chemical communication research, realization of the importance of infant and adult experience in mammalian responses to odors grew quickly (Marr and Gardner, Jr., 1965; Mainardi, Marson, and Pasquali, 1965; Carr, Loeb, and Dissinger, 1965). For example, Mainardi et al. (1965) found that female mouse pups reared with artificially perfumed parents preferred adult perfumed males upon adulthood, suggesting that sexual preferences may be influenced by early learning of the parents' characteristics. Mammalian behavior appears to be more dependent on experience and consequently more variable than insect behavior. This is consistent with the fact that there exists a much greater degree of encephalization in mammals than in insects. Because of such apparent response variability, Bronson (1968) suggested that the category of "releaser" pheromone be amended to "signalling" pheromone. The signalling pheromone would provide information to the recipient but such

information may or may not lead to a change in the recipient's behavior. The behavioral response would depend more upon previous infant and/or adult experience than had originally been anticipated from insect communication research.

Further research in mammalian communication not only confirmed the fact that olfactory cues are usually not used exclusively as determinants of a particular behavioral response, but also demonstrated that context is crucial in determining the particular response. For example, if the vaginal secretions of a female golden hamster are applied to a white clay model of a hamster, males will investigate but will not respond sexually. If the secretions are applied to an anesthetized male hamster, then it is apparently mistaken for a female and sexual behaviors are aroused (Devore and Murphy, 1972). Certain contextual factors, including odors, need to be present for intra-specific communication to be effected. Therefore, it appears that there exists no obvious place to draw the line between the "pheromone" itself and other information-transferring signals (Beauchamp et al., 1976).

Because of the plasticity of mammalian behavioral response and the apparent chemical complexity of supposed pheromonal odors, Bronson (1974) advised that the pheromone

concept be used only where natural functions are obvious and easily definable, and where it seems reasonable to predict isolation and identification of a discrete compound or at most a restricted mixture of compounds. Bronson's advice was clearly a call to return to the original criteria established for pheromones as being the only way the concept would retain its heuristic value for mammalian chemical communication research.

Beauchamp et al. (1976) went one step further and called for an abandonment altogether of the pheromone concept as applied to mammalian chemical communication research. They argued that if learning factors and not genetic factors commonly underlie response variability in mammals, then the pheromone concept adopted from insect communication research is not appropriate for describing mammalian chemical communication. Though some mammalian odorants with signalling properties had been isolated and identified chemically, social context factors had not been systematically examined (Beauchamp et al., 1976).

Mouse Ultrasounds and the Pheromone Concept

One line of mammalian chemical communication research which originally appeared to fit the criteria for pheromonal communication was the male mouse (*Mus musculus*) ultrasonic response to female odors. Ultrasounds are used by a variety of myomorph rodents for intra-specific

communications such as alarm, aggression, and courtship (Sales, 1972). However, in male mice, ultrasounds occur mainly in encounters of a sexual nature (male-sniff-male; male-mount-female) (Sales, 1972). In Mus musculus, a previously isolated male will emit ultrasounds when placed together with a female, though ultrasounds are not detected in a variety of other situations such as gentle and rough handling, dropping, pushing over a precipice, restraint, and electric shock (Whitney, Coble, Stockton, and Tilson, 1973).

Since ultrasonic emissions correlate so well with a naturally occuring behavior, i.e., sexual behavior, and are so well-defined, some researchers decided to use this behavioral response as an indicator of sexual responsiveness and a measure of the signalling value of various odoriferous stimuli (Nyby and Whitney, 1978b). At that time, it seemed that ultrasonic vocalizations in response to female odorants conformed to the criteria for pheromonal communication research.

Whitney, Alpern, Dizinno, and Horowitz (1974) performed a series of studies on the biochemical elicitation of male ultrasounds. They found that cage shavings from female occupied cages elicited ultrasounds from males, and that the emission of these ultrasounds was not eliminated by preventing tactual contact with the shavings, though

the frequency of emission was lowered. Female odors in the shavings apparently signalled the concept "female" to the males. Nyby, Wysocki, Whitney, and Dizinno (1977) also found that biochemicals such as urine, vaginal secretions, and facial substances elicited ultrasounds from males, while corresponding male and control substances were not effective in ultrasound elicitation.

Interestingly, some of the early studies (Whitney et al., 1973, 1974) concerned with the biochemical elicitation of male mouse ultrasounds did not make an explicit conceptual or experimental distinction between conditioned and unconditioned stimuli. The first experiment to attempt to take into consideration a possible role for experience made a distinction between "socially experienced" and "socially naive" male mice (Nyby et al., 1977). However, the supposedly naive males had been given one three-minute exposure to the social experience females to familiarize them with the test situation. Apparently, the role of experience was not yet fully appreciated.

Dizinno, Whitney, and Nyby (1978), in another urinary cues experiment, explicitly discuss the role of experience in the biochemical elicitation of male mouse ultrasounds. Dizinno et al. (1978) redefined "socially naive" as total separation from females at weaning. In this experiment, socially naive males failed to emit detectable ultrasounds

when exposed to female urine alone. Dizinno et al. (1978) further found that while naive adult males did not emit ultrasounds to female urine alone upon first exposure, they did emit ultrasounds to females upon first exposure. Thus the male's response to the female herself was not learned in adulthood.

Some findings had further suggested that sexual odors are learned in a critical imprinting period during infancy (Marr and Gardner, 1965; Muller-Schwarze, 1974). So in another experiment by Nyby, Whitney, Schmitz, and Dizinno (1978a), the role of infant and adult experience upon ultrasonic emissions was examined to determine if there was a critical imprinting period for mice.

In this experiment, males were assigned to four different odor treatment groups. The first group had perfume sprayed on both the mother and the adult, social-experience female (P-P group). The second group had perfume sprayed on the mother; ethanol was used on the adult social-experience female to control for the spraying procedure (P-E group). The third group had perfume sprayed on the adult social-experience female, while ethanol was sprayed on the mother (E-P group). The fourth group had ethanol sprayed on both the mother and the adult social-experience female (E-E group). All male subjects were housed with the parents until 21 days of age, then housed in single-sex

littermate groups. Between 46 and 73 days, all subjects were individually housed. Social experience followed, consisting of one 3-minute exposure each day to the appropriate male and female social-experience animals for eight consecutive days.

Artificial perfume does not normally elicit ultrasounds from male mice since males without prior perfume exposure emitted few ultrasounds to perfume (E-E group). However, the two groups that encountered adult female mice odorized with perfume emitted ultrasounds in response to perfume itself (P-E and E-P groups). This finding indicates that some artificial odors can become conditioned stimuli having signalling value. However, when both mother and social-experience female had been sprayed with perfume (P-P group), males showed a greater response to the perfume stimulus than males for which the adult females alone received spraying with perfume (E-P group). Thus, preweaning exposure seemed to further potentiate the adult learning effect.

There are several possible interpretations of how male ultrasonic response to females and female urine develop. First, some aspect of the female's phenotype may be the unconditioned stimulus while female urine is the conditioned stimulus. Second, urine may be an unconditioned stimulus only when it is present on the animal (Nyby et al., 1978a). It would be this stimulus-complex

that would constitute the "signalling pheromone." Whitney et al. (1973) reported that since males did emit ultrasounds to an anesthetized female, it was unlikely that some active component of female behavior was important in eliciting ultrasounds. However, when this experiment was performed, social-experience had not been considered a relevant variable to be manipulated. Consequently, the odors could have been conditioned stimuli and some active component of female behavior could previously have been involved in causing these odors to acquire signalling value.

Concluding Remarks

To date, mammalian chemical communication research suggests that certain infant and adult experiences are necessary for some odors (conspecific or artificial) to be preferred or avoided (for example: house mice: Caroom and Bronson, 1971; Hayashi and Kimura, 1974; spiny mice: Porter and Etscorn, 1975; Porter and Doane, 1976; deer: Muller-Schwarze and Muller-Schwarze, 1971; dogs: Doty and Dunbar, 1974). However, such preference studies do not allow strong inferences concerning the signalling value of the odors. Where there is clear signalling value to odoriferous stimuli, the criteria variously used to identify pheromonal communication are not well met (Whitney et al., 1974; Nyby et al., 1977; Dizinno et al., 1978). For example, in mouse ultrasound communication research, the

chemical complexity of urine and the apparent dependency of the male mouse sexual response upon previous experience do not readily conform to the criteria variously used to identify a chemical substance as pheromonal. To label odors involved in olfactory communication as "pheromonal" when they have not been demonstrated to meet a well-defined set of operational criteria is problematic if the pheromone term is to have any meaning beyond that of being synonymous with a chemical communicator (Beauchamp et al., 1976). It may be that we should be discussing "olfactory signals" and "pheromones" in mammals, the use of the latter term being restricted to compounds evoking neuroendocrine responses (the classical primer pheromone).

In spite of these problems with the pheromone concept, the term seems firmly entrenched in the minds of researchers (Brown, 1977; Breen and Leshner, 1977; Porter and Doane, 1976; Leon, Bennett, and Behse, 1977). The term will probably remain in the literature for quite some time (Bronson, 1976).

CHAPTER 2

General Method

Introduction

In previous mouse chemocommunication research, female-soiled cage shavings, vaginal secretions, female facial substances, perfume appropriately paired with females, and female urine, were found to elicit ultrasounds from males. Further, female urine did not elicit ultrasounds from adult males who had received no previous exposure to either adult females or males.

The following two experiments systematically assessed the effect of exposure to adult females and males in the development of the differential response to male and female urine by adult males. More specifically, the following concerns were explored:

1. The possibility that exposure to urine would be sufficient for the development of ultrasound elicitation by urine;

2. The number of times a male must be exposed to a female for the development of the ultrasonic response to urine;

3. The ability to experimentally alter the normal signalling value of male and female urine.

The following experiments were based on the hypothesis that the ultrasonic response to male and female urine fits a classical conditioning paradigm. In the paradigm, as applied to the concerns of these experiments, the unconditioned response (UCR) is the ultrasonic response of an adult male mouse to another adult male or female mouse, which is the unconditioned stimulus (UCS). The conditioned stimulus (CS) is male or female urine with the ultrasonic response of the male mouse to the urine stimulus as the conditioned response (CR).

The experiments involved the following groups of animals: a control group, homotypical treatment group, heterotypical treatment group, social-experience animals, and urine-donor animals.

In the control group, male mice were presented with male and female urine stimuli alone (urine was presented on a clean cotton swab). This was designed to check for the possibility that exposure to urine alone would be sufficient for the development of ultrasound elicitation by urine.

The social-experience animals were adult males and females which served as unconditioned stimuli (UCS) in the homotypical and heterotypical treatment regimes. Urine donor mice were used to provide the male and female urine for urine swab stimuli serving as (potentially) conditioned stimuli (CS).

In the homotypical group, adult male mice received exposure to other adult males and females (the UCS) which were preceded by urine stimuli (the CS) of the same sex as the social-experience animal. This was designed, in part, to determine the number of times a male mouse must be exposed to a female social-experience animal for the development of the ultrasonic response to urine.

In the heterotypical group, adult male mice received exposure to other adult males and females (the UCS) which were preceded by urine stimuli (the CS) of the sex opposite to that of the social-experience animal. This was an attempt to experimentally alter the normal signalling value of male and female urine (the CS).

The general procedures used in both experiments will be described. Any deviation or additions from these will be described in the individual experiments.

Animals

The male subjects were experimentally naive DBA/2J (DBA) adult males obtained from Jackson Laboratories (Bar Harbor, Maine). DBA's were chosen because it has been shown that a high proportion of adult males from this strain emit ultrasounds to female urine after being paired with a social-experience female (Nyby et al., 1978b). C57BL/6J (C57) adult males and C57 adult females served as the social-experience animals. C57's were

chosen as social-experience animals because they are black and easily distinguished from DBA's which are gray. Further, a high proportion of adult C57 males are low ultrasound emitters. Using C57's helps to insure that the majority of ultrasounds monitored during male-male social-experience pairings are coming from the DBA male subject.

C57 males and C57 females served as urine donors. More females than males were required because females are smaller than males and produce less urine during the night (given food and water ad lib).

Apparatus

Ultrasounds were detected with a QMC receiver, set to a center frequency of 70 kHz. The receiver transforms ultrasonic vocalizations into low frequency audible sounds which can be monitored by an observer who is blind to subject and stimulus identity. This receiver works by mixing the microphone signal with another wave form from an internal tunable oscillator so that a new signal is produced at a frequency equal to the difference of the first two. Thus ultrasound frequencies are effectively translated down to any desired audible frequency by adjusting the tuning knob (Sales and Pye, 1974). To use this detector, it is not necessary to understand its principles, only the way in which it behaves. The receiver responds only to ultrasounds within a narrow frequency band, about

10 kHz wide, which can be tuned by a knob controlling the internal oscillator. The microphone was located about 14 cm above the test chamber. The test chamber consisted of each subject's home cage during all phases of the experiment.

Urine was collected in two E110 metabolism units (Maryland Plastics). The urine was drawn from the collection vial of the metabolic cage into 1.0 ml syringes. Cotton-tipped surgical swabs were used to present the urine to the subjects.

Social-experience animals were individually housed in 24 cm x 20 cm x 18 cm metal cages containing a wire mesh bottom. Subjects were individually housed in 18 cm x 28-1/2 cm x 12-1/4 cm plastic cages with wood shavings for bedding. Urine donors were group housed in 24 cm x 20 cm x 18 cm metal cages. Male urine donors were housed together. Female urine donors were housed in two groups. Food and water were available ad libitum for all animals.

Procedure

Upon arrival in our lab, both subjects and social-experience animals were individually housed. Urine donors were group housed at this time. All animals were maintained on a 12-12 light-dark cycle, with lights going on at 6 a.m. Urine was collected during the night preceding its use by housing the urine donors by sex in the metabolism

units. Food and water were available ad libitum during urine collection periods. After 12 hours in the metabolism units, the urine donors were returned to their home cages. The metabolism units were thoroughly cleaned with detergent and hot water between collection periods.

Urine was collected on the morning prior to its use into 1.0 ml syringes at approximately the same time prior to testing. Two syringes of male urine and two of female were prepared, each containing 1.0 ml of urine. A stimulus was prepared by placing 0.1 ml urine of the appropriate sex on a cotton swab immediately prior to testing of each subject.

Before testing, all food and water were removed from subjects' home cages, and the cage tops were turned upside down to permit greater freedom of movement for the animals. When testing began, the subject was transferred from its rack to a table in the testing room. The subject was placed under the ultrasonic monitoring microphone, and monitoring of ultrasounds began immediately.

Figure 1 on page 24 illustrates the sequence of behavioral testing for both experiments. Each subject went through the sequence twice per day. The sequence began with each subject being given a 1-min habituation period. If ultrasounds occurred during this period, then 2-min were required to elapse without an ultrasound prior to the next test phase. The habituation period was used to insure

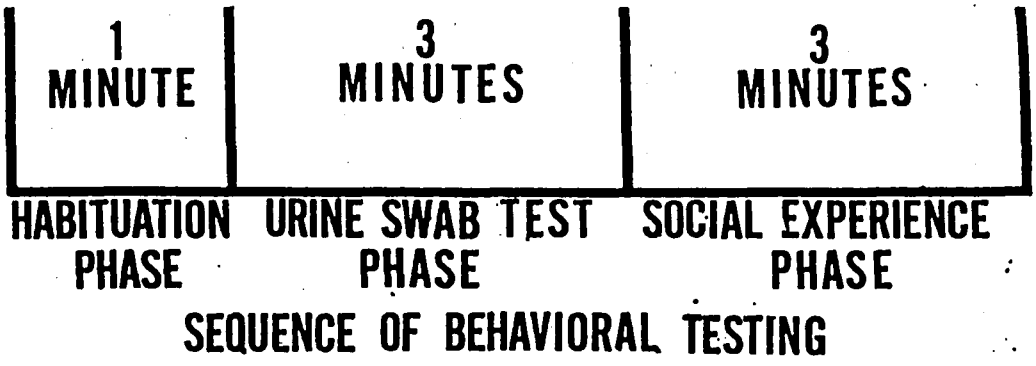

Figure 1: Sequence of Behavioral Testing used in both Experiment 1 and 2.

that the animals were not responding to incidental aspects of the experimental situation. Upon completion of the habituation period, the subject received a 3-min exposure to a swab containing 0.1 ml of the appropriate urine. The swab was prepared immediately prior to testing, during the habituation period. The swab was presented by placing the cotton end of the swab into a clean test tube, breaking off the portion touched by the experimenter, and "pouring" the remainder into the subject's cage. This procedure was to insure that the swab "poured" into the subject's cage had only urine odors on it.

The 3-min exposure to urine was followed by the appropriate social-experience condition for a duration of 3-min. Exposure to a social-experience animal involved moving the subject animal in his home cage to the counter about 10 feet away from the ultrasonic microphone in order to insure that ultrasounds emitted during social-experience were not picked up by the microphone, which was monitoring ultrasounds for the next subject. A stimulus animal was then placed into the subject's home cage. Exposure continued for 3-min or until the male subject and a male stimulus animal began fighting. If fighting occurred the stimulus animal was immediately removed (such aggression occasionally occurred between two males). Subjects not receiving social-experience with stimulus animals were removed from the table and placed in the same

location as those subjects receiving social-experience exposures, but did not receive a stimulus animal. After the social-experience condition, animals were put back on their racks.

Ultrasounds were monitored during habituation and urine swab test phases. Ultrasounds were quantified during swab tests by dividing the 3-min test into 36 5-sec blocks and counting the number of blocks containing ultrasounds.

The subject order for testing was randomized daily. Since subjects went through two sequences each trial, thereby receiving both male and female urine swabs for that day, the random order selected for the first sequence was repeated for the second, to equate the interval between sequences for all subjects. For each animal the order across trials of presentation of male and female urine swabs was alternated daily. The pairing of a particular social-experience animal with a particular subject was random across days.

An analysis of variance was used to analyze the results of both experiments. All data were changed by a square root transform in order to more closely approximate the assumption of the homogeneity of variance required for analysis. (Tables are located in the back.)

CHAPTER 3

Experiment 1

Previous work (Dizinno et al., 1978) suggested that the sex-signalling value of female mouse urine is learned. For example, exposure to females was necessary before female urine elicited ultrasounds from males. In addition, continued presentation of female urine to males, in the absence of the female herself, caused the urine to lose its ultrasound eliciting potency. Finally, while the signal value of urine appears to be learned by adult males, the signal value of the female does not. Adult males emit ultrasounds upon first exposure to a female in adulthood. All of these findings are consistent with a classical conditioning interpretation with female urine being a conditioned stimulus for ultrasound elicitation and some other aspect of the female being an unconditioned stimulus.

It remained possible, however, that males "know" the signal value of female urine but that repeated exposures (sensitization) are necessary before males emit ultrasounds to urine. Thus, in this first experiment, it was of interest to determine whether repeated exposures to female urine, in the absence of the female, would allow female urine to take on ultrasound eliciting properties.

Specifically, the following questions were asked:

1. Will urine take on ultrasound eliciting properties if the naive male receives exposure to male and female urine, but no social-experience with either males or females;
2. How many times must a socially naive male be exposed to other animals before female urine comes to elicit ultrasounds.

Method

Animals

Twenty DBA males were used as subjects. Five C57 adult males and 5 C57 adult females served as the social-experience animals. Three additional C57 males and 6 females served as urine donors. At the start of the experiment, DBA male subjects were 58 days of age and C57 social-experience animals and urine donors were 123 days of age.

Procedure

Prior to testing, all 20 subjects were randomly assigned to either a control (N = 10) or experimental-homotypical (N = 10) treatment group. For the homotypical treatment group, presentation of a social experience animal was immediately preceded by a urine stimulus collected from the urine donors of the same sex as the social-experience animal. (This is in contrast to the heterotypical treatment group used in experiment 2 in which presentation of a social-experience animal was preceded by a urine stimulus

collected from urine donors of the sex opposite to that of the social-experience animal). For one sequence each trial the social-experience animal was male, while for the other sequence, the social-experience animal was female. For the control group, both male and female urine stimuli were presented but not followed by social-experience animals.

The social-experience regime proceded for eight consecutive days so that results would be comparable to earlier studies (Dizinno et al., 1978; Nyby et al., 1978a). On the ninth day, a post-test was done in which all subjects were monitored for their ultrasonic responsiveness to male and female social-experience animals.

Results

Urine Swab Test Phase

The results of this experiment for the urine swab test phase are seen in Figure 2 on page 30. Consistent with the results of previous mouse ultrasound research, exposure to males and females caused female urine to acquire ultrasound eliciting ability. However, the group that received no exposure to males or females emitted virtually no ultrasounds to either male or female urine. These results are reflected in both the significant difference in the amount of ultrasounds emitted by the two treatment groups ($F(1,18) = 18.13$; $p < .01$) and in the significant interaction between

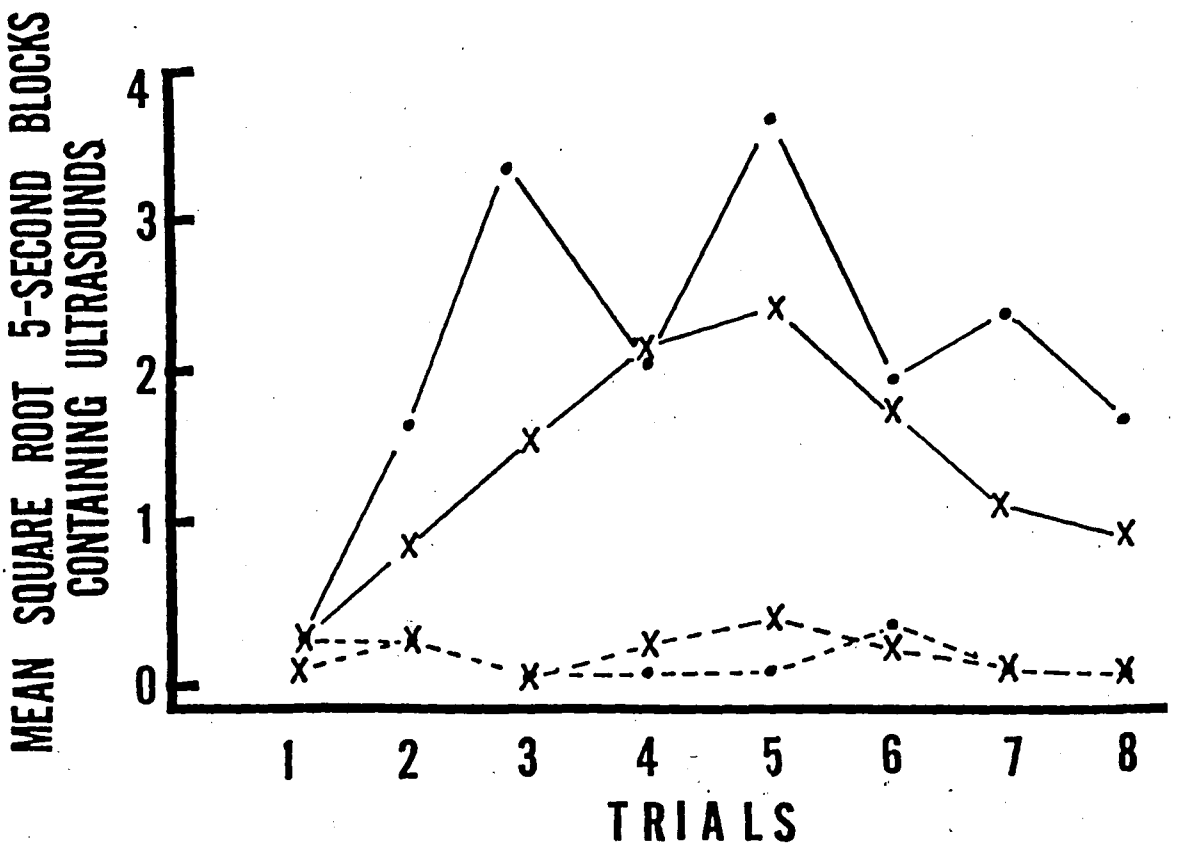

Figure 2: Mean of the square root of the frequency of 5-second blocks containing ultrasounds for control group (receiving no social experience) and the homotypical group (receiving social experience). The conditions graphed above include the control males' response to male urine (X----------X) and female urine (●----------●), and the homotypical males' response to male urine (X——————X) and female urine (0——————0).

treatment condition and trials ($F(7,126) = 4.20$; $p < .01$). Since the control group emitted virtually no ultrasounds, while the homotypical treatment group emitted many ultrasounds, the assumption of the homogeneity of variance and the normality of distribution was violated. Therefore, a Mann-Whitney U test was also done comparing the homotypical group's total ultrasonic response to urine to the control group's total response to urine. There was a significant difference (Mann-Whitney U, $U = 0$, $p < .001$) between the groups, supporting the results of the analysis of variance. Given that virtually no ultrasounds were emitted by the control group and the significant results of these statistical tests, it appears that exposure to female urine is not sufficient to cause urine to acquire ultrasound eliciting ability; the female urine must be paired with a female.

There was no significant difference between ultrasonic response to male urine swabs and ultrasonic response to female urine swabs ($F(1,18) = 2.64$; N.S.). Further, a correlated t-test comparing the mean responses of the homotypical animals to female and male urine swabs was not significant ($t(9) = 1.78$; N.S.). Also, there were no significant treatment X sex-urinary stimulus ($F(1,18) = 2.84$; N.S.), trials X sex-urinary stimulus ($F(7,126) = 1.15$; N.S.), or treatment X trials X sex-urinary stimulus

$F(7,126) = 1.82$; N.S.) interactions. Thus in contrast to previous work (Dizinno et al., 1978), the male subjects did not appear to be discriminating male and female urine.

This experiment allowed us to examine the time course for this biologically relevant learning. As can be seen in Figure 2 on page 30, exposure to male and female urine swabs for 8 consecutive days caused a significant change in ultrasonic response across the 8 trials ($F(7,126) = 4.28$; $p < .01$). Only two or three exposures to female urine were sufficient for female urine to acquire maximum ultrasound eliciting potency. The apparent ease with which males learn to respond to urine suggests that males are biologically predisposed to learn the signal value of urine, although it would be of interest to establish the time course for some artificial odors with which the time course for urine could be compared. Further, the homotypical group's response to both male and female urine swabs began to decline after the fifth trial, suggesting that the animals were beginning to discriminate between the urine swab stimuli and the animal stimuli.

Male urine swabs acquired some ultrasound eliciting ability, reaching its maximum potency also after two or three exposures to male urine. It may be that the animals were responding to the cotton swab itself since the swab was associated with both male and female urine. Alternatively, male urine may be sufficiently like female urine to

facilitate some sexual arousal. However, further experimentation with appropriate controls would have to be done to clarify the reason for male subjects' response to male urine swabs.

Post-Test with Animal Stimuli

The results of the post-ultrasonic tests with social-experience stimuli are given in Figure 3 on page 34. Post-test scores were in response to male and female social-experience animals. As can be seen, there was a significant difference between the amount of ultrasounds emitted to males and the amount emitted to females ($F(1,28) = 212.38$; $p < .01$). Further, there was a significant treatment X sex-stimulus animal interaction ($F(1,28) = 49.21$; $p < .01$). This interaction reflects a lower amount of ultrasounds emitted to males by the homotypical group, which received social-experience, compared to the control group which received no social-experience until the post-test itself. This finding suggests that some ability to discriminate between male characteristics and female characteristics is learned. There was no significant difference between the two treatment groups in their ultrasonic response to the social-experience stimuli ($F(1,8) = 2.97$; N.S.).

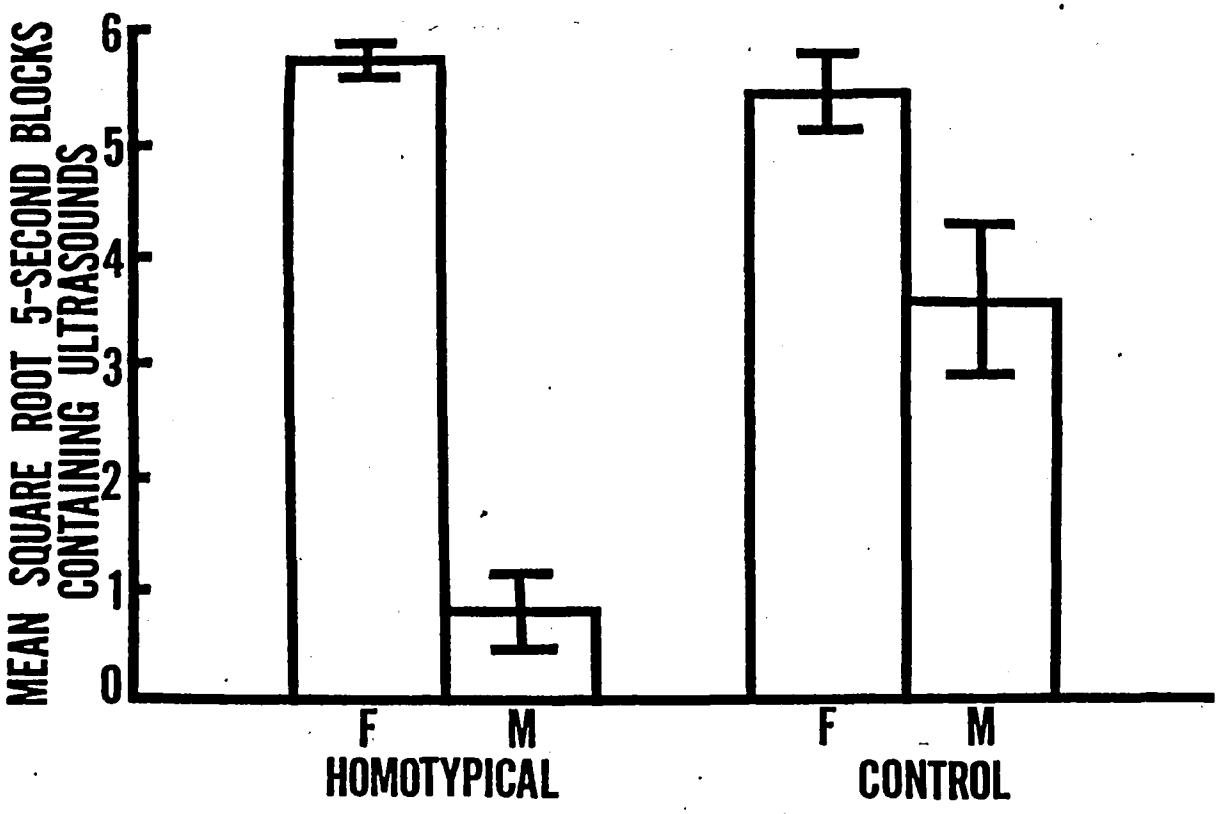

Figure 3: Mean of the square root of the frequency of 5-second blocks (± SE) containing ultrasounds for post-test scores in response to male and female social experience animals. The post-test score was recorded the day after acquisition was terminated, i.e. day 9. In the above figure, "M" = male stimulus animal, "F" = female stimulus animal.

CHAPTER 4

Experiment 2

Some research (Nyby et al., 1977; Nyby et al., 1978a) indicated that male ultrasonic response to female cues is not restricted to female urine. Female facial cues, vaginal cues, and even artificial odor (Wild Musk Spray, Coty) which is appropriately associated with a female, will elicit ultrasounds. Thus the potential of odors to signal "female gender" may not be tightly constrained in terms of either natural or artificial odors.

In this second experiment, possible constraints upon learning the signal value of male and female mouse urine, as well as a number of other considerations, were further examined. Specifically, the following questions were asked: 1. Can the signal value of male and female urine be altered by pairing urinary cues with social-experience animals of the opposite sex; 2. How many times must a socially naive male be presented with a female before female urine comes to elicit ultrasounds; 3. Once female urine has acquired ultrasound eliciting potency, how many times must the urine be presented without pairing it with a female before it loses ultrasound eliciting potency; and 4. Will spontaneous recovery occur after extinction?

Method

Animals

Twenty DBA adult males were used as subjects. Five C57 adult males and five C57 adult females served as the social-experience animals. Three additional C57 adult males and six C57 adult females served as urine donors.

At the start of the experiment DBA male subjects were 60 days of age and C57 social-experience animals were 87 days of age.

Procedure

Prior to testing, all 20 subjects were randomly assigned to either a "homotypical" or "heterotypical" treatment group. For the homotypical treatment group, presentation of a social-experience animal was immediately preceded by a urine stimulus collected from the urine donors of the same sex as the social-experience animal. For the heterotypical treatment group, presentation of a social-experience animal was preceded by a urine stimulus collected from urine donors of the sex opposite to that of the social-experience animal.

On the first day of acquisition (trial 1), ultrasounds were monitored during all three phases of behavioral testing and quantified during the urine swab and social-experience test phases. Ultrasounds were monitored during the social-experience phase to determine the subjects'

response to the social-experience animals. Subsequent test days involved monitoring ultrasounds during the habituation and swab phases of the test, and quantifying ultrasounds during the swab test phase.

The acquisition testing phase proceeded for 8 days so that results would be comparable to earlier studies (Experiment 1 of this thesis; Dizinno et al., 1978; Nyby et al., 1978a). On the 9th day, a post-test with the original animal stimuli was done for all subjects to determine the subjects' ultrasonic response to the social-experience animals.

Starting on the 11th day, the subjects were given 3-min exposures to both male and female urine swabs as before, but without being followed by presentations of social-experience animals. Ultrasounds were monitored until extinction occurred. Extinction was defined as a level of ultrasonic responsiveness to females below the highest level of the subject animals' ultrasonic responsiveness to males during learning. The extinction phase lasted 12 days.

Three days after the extinction phase was terminated subjects were again tested for ultrasonic responsiveness to male and female urine swabs, to see if "spontaneous recovery" would occur. Another post-test with social-experience stimuli was done to determine the amount of

ultrasonic response to the social-experience animals on the day following the spontaneous recovery test.

Results

Urine Swab Test Phases

Acquisition

As can be seen in Figure 4 on page 39, in contrast to Experiment 1 and in agreement with previous work (Dizinno et al., 1978), a significant difference existed between the amount of ultrasounds emitted in response to male urine and the amount emitted in response to female urine ($F(1,18) = 129.32$; $p < .01$). Consistent with the results of Experiment 1, exposure to male and female urine swabs for 8 consecutive days caused a significant change in ultrasonic response across the 8 trials ($F(7,126) = 11.12$; $p < .01$. Further, the heterotypical group's ultrasonic response to male urine swabs and the homotypical group's responses to male and female urine swabs declined after the fifth trial. The heterotypical group's response to female urine declined after the seventh trial. Again, it may be that subject animals were learning to discriminate between urine swab stimuli and animal stimuli. As in Experiment 1, the ultrasonic response to the female urine swab reached its maximum after only two 3-min exposures to the social-experience animals. Also consistent with Experiment 1, male urine swabs acquired some ultrasound

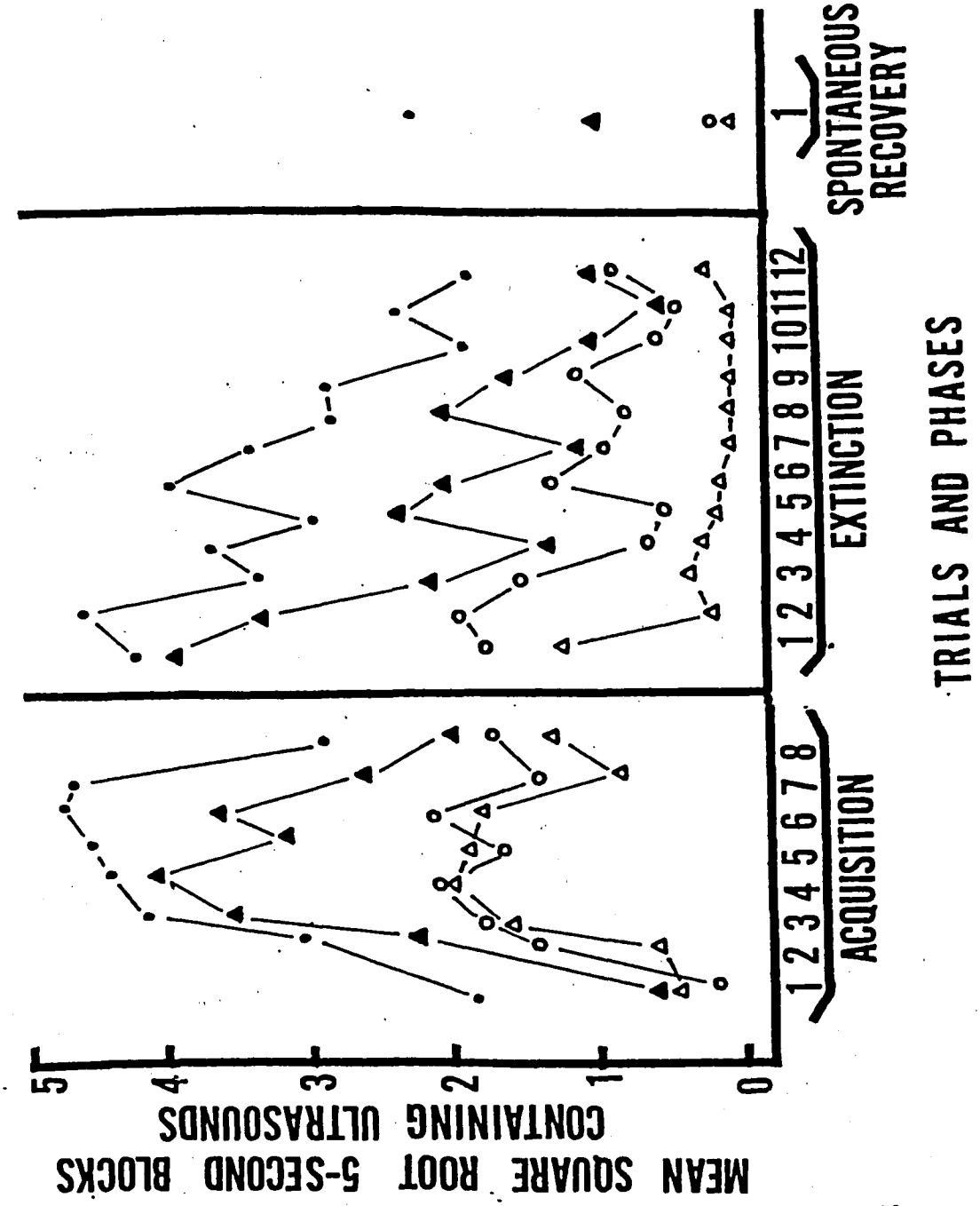

Figure 4: Mean of the square root of the frequency of 5-second blocks containing ultrasounds for homotypical and heterotypical groups in response to male urine and female urine during acquisition, extinction, and spontaneous recovery phases. The conditions graphed above include the homotypical males' response to male urine (△———△) and female urine (0———0) and the heterotypical males' response to male urine (▲———▲) and female urine (●———●).

eliciting ability, reaching its maximum potency after two or three exposures to the social experience animals.

The heterotypical treatment condition, involving differential sequential pairings of urine with stimulus animals, was not able to alter the signal value of male and female urine. This was probably due to design problems based upon repeated measures. There was no significant difference in the amount of ultrasounds emitted between the homotypical and heterotypical treatment groups ($F(1,18) = 1.92$; N.S.). Further, there were no significant treatment X trials ($F(7,126) = .47$; N.S.), trials X sex-urinary stimulus ($F(7,126) = 1.95$; N.S.), and treatment X trials X sex-urinary stimulus ($F(7,126) = .39$; N.S.) interactions. However, a significant treatment X sex-urinary stimulus interaction ($F(1,18) = 5.68$; $p < .05$) indicated a significantly greater difference in response between male and female urine by the heterotypical group than by the homotypical group. This treatment X sex-urinary stimulus interaction reflects a greater response to female urine by the heterotypical group than the homotypical group, since the response to male urine by both groups was approximately the same.

Extinction

During the extinction phase, the ultrasonic response to male and female urine declined across trials ($F(11,198)$

= 8.81; $p < .01$), thus replicating earlier findings (Dizinno et al., 1978). A significant difference in ultrasonic response to male and female urine swabs ($F(1,18) = 77.30$; $p < .01$) was maintained in extinction. Also, there was a significant difference between the homotypical group's responses to male and female urine and the heterotypical group's response to urine ($F(1,18) = 4.57$; $p < .05$). A floor effect for the ultrasonic response to male urine was reflected in the significant interactions between sex-urinary stimulus and trials ($F(11,198) = 24.37$; $p < .01$). Further, the level of ultrasonic response to female urine swabs for both the homotypical and heterotypical groups is higher at the start of the extinction phase than at termination of the acquisition phase. Three days separated termination of acquisition and the beginning of extinction; therefore, the increase in ultrasonic response at the start of extinction may be a spontaneous recovery phenomenon.

There were no significant treatment X trials ($F(11,198) = 96$; N.S.), treatment X sex-urinary stimulus ($F(1,18) = .61$; N.S.) and treatment X trials X sex-urinary stimulus ($F(11,198) = .81$; N.S.) interactions.

Spontaneous Recovery

An attempt to demonstrate the phenomenon of spontaneous recovery after the extinction phase had been

terminated, also seen in Figure 4 on page 39, was not successful.

Post-Test with Animal Stimuli

The results of the pre- and post-ultrasonic tests with original stimuli are given in Figure 5 on page 43. As can be seen, there was a significant difference between the amount of ultrasounds emitted in response to males and the amount emitted in response to females ($F(1,18) = 75.46$; $p < .01$). Also, there was a significantly less amount of ultrasonic response to males by the experimental male subjects after repeated exposures to social-experience animals than before the social-experience exposures, (reflected in the significant sex-stimulus animal X test interaction ($F(2,36) = 17.86$; $p < .01$)). Consistent with the results of the post-tests in Experiment 1, this significant decline in response to male stimuli by male subjects suggests that some ability to discriminate male characteristics from female characteristics is learned.

There were no significant differences in ultrasonic response to original animal stimuli between the two groups ($F(1,18) = 44$; N.S.) nor among the pre- and two post-tests ($F(2,36) = 1.66$; N.S.). Further, there were no significant treatment X sex-stimulus animals ($F(1,18) = .28$; N.S.), treatment X test ($F(2,36) = .053$; N.S.), nor treatment X test X sex-stimulus animal ($F(2,36) = .021$; N.S.) interactions.

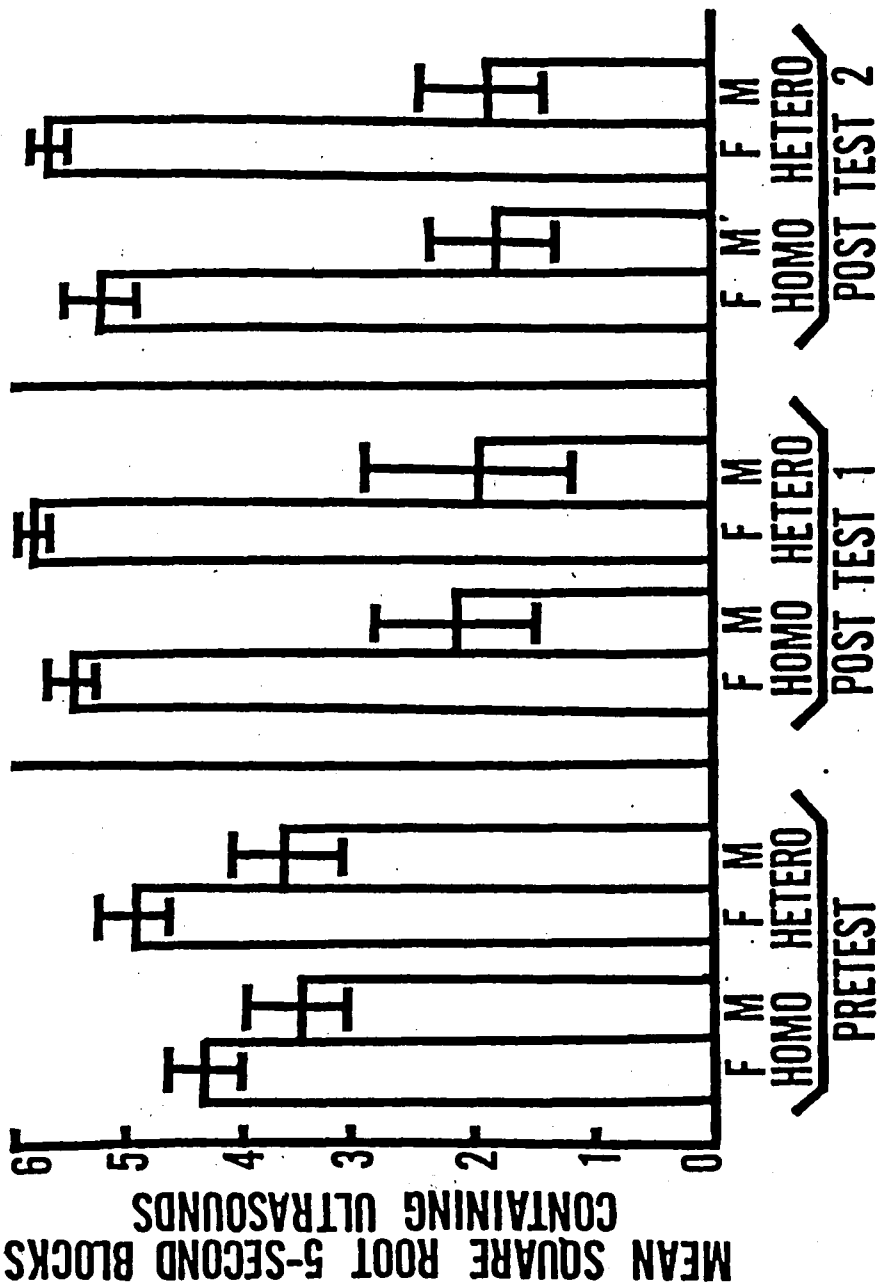

Figure 5: Mean of the square root of the frequency of 5-second blocks (± SE) containing ultrasounds for pre- and post-test scores in response to male and female social experience animals. The pre-test score was recorded on the first day of acquisition. Post-test #1 was recorded on the day after acquisition was terminated. Post-test #2 was recorded on the day after the spontaneous recovery test. In the above figure, "M" = male stimulus animal, "F" = female stimulus animal, "Homo" refers to the homotypical group, and "Hetero" refers to the heterotypical group.

CHAPTER 5

Discussion

Urine Swab Test Phases

Acquisition

Previous work (Dizinno et al., 1978) suggested that the sex-signalling value of female mouse urine is learned. The above experimental findings are consistent with the results of this research, and suggest that both the acquisition and maintenance of the male ultrasonic response to female urine are influenced by experiential factors. The two experiments reported here indicate that an adult male requires some experience with adult conspecifics before female urine acquires its ultrasound eliciting potency; sensitization to urine alone is not sufficient for ultrasound elicitation. Specifically, only two or three 3-min exposures to a female were sufficient for female urine to acquire full ultrasound eliciting potency. This result suggests that males may be biologically predisposed to learn the signal value of female urine.

Although males may be biologically predisposed to respond ultrasonically to female urine, previous research indicated that ultrasound eliciting properties are not constrained to only naturally occurring biological odors (Nyby et al., 1977; Nyby et al., 1978a). A "musk-like"

perfume, for example, could acquire ultrasound eliciting ability if appropriately paired with a female. However, attempts to increase the ultrasound eliciting potency of male urine and decrease the potency of female urine by pairing the presentation of the urine swab with social-experience animals of the opposite sex were not successful. The inability of the treatment conditions of Experiment 2 to alter the signal value of male and female urine may be due to the following conditions. In the heterotypical group, a social-experience animal was paired with a urine swab of the opposite sex. However, the social-experience animal undoubtedly had its own urine on its body from grooming. (Grooming involves the animal "washing" its genitalia, body and head with its front paws). Therefore, the experimental male subject was in effect receiving exposure to a social-experience animal paired with the same sex urine. Thus the exposure to social-experience animals that the heterotypical group received was identical to that received by the homotypical group. This similarity of treatment conditions might explain the similarity of responses by the two experimental groups to both male and female urine swabs.

One experimental design that might answer the question whether or not the signal value of male and female urine can be altered is the following. Since the evidence

indicates that the ability of urine to elicit ultrasounds is acquired and that males learn to discriminate between male and female urine, it would be appropriate to use two treatment groups, limiting each group to one social-experience condition only. Each male subject in one treatment group would be given social-experience with a female "painted" with male urine, while each male subject in the other treatment group would be given social-experience with a male painted with female urine. In this case, the mixing of odors is not relevant; only the pairing of females with male urine and males with female urine is important. Both groups of animals would then be tested for their ultrasonic response to male and female urine swabs. If male urine swabs came to elicit ultrasounds from males given social-experience with females painted with male urine, and female urine swabs did not elicit ultrasounds from males given social-experience with males painted with female urine, then the hypothesis that the signal value of male and female urine can be altered would be supported. Clearly the constraints upon ultrasound elicitation by odoriferous stimuli need further exploration.

In Experiment 1, there was no significant difference between the ultrasonic response to male urine swabs and the response to female urine swabs by the homotypical group. This result was not expected, and is not consistent with

the results of the acquisition phase of Experiment 2. It may be that the lack of a significant difference between the ultrasonic response to male urine and the response to female urine by the homotypical group was due to a floor effect. The ultrasonic response to female urine was low, compared to the ultrasonic response to female urine by the treatment groups in Experiment 2. If the ultrasonic response to female urine is low, it would be difficult to demonstrate a significant difference between male and female urine. The low ultrasonic response to female urine by the homotypical group of Experiment 1 may be a chance occurrence.

In both experiments, there was some ultrasonic response to male urine swabs. It may be that the cotton swab became associated with receiving social-experience with a female stimulus animal and thereby took on some ultrasound eliciting ability. Alternatively, it may be that male urine is sufficiently like female urine to facilitate some sexual arousal. However, further experimentation with appropriate controls would have to be done in order to determine the reason for male urine swabs taking on some ultrasound eliciting ability.

As already noted for the acquisition phase of Experiment 2, there was a significant treatment X sex-urinary stimulus interaction, which reflects a greater response to

female urine by the heterotypical group than by the homotypical group. Upon closer examination of the data, it appears that the heterotypical treatment regime was responsible for this effect. For the first sequence of trial 1, some of the subject animals in the heterotypical group were given exposures to male urine swabs followed by females. As suggested above, females probably had female urine on their bodies from grooming. Those heterotypical male subjects exposed to male urine swabs followed by female stimulus animals were thereby exposed to female urine. Thus, for the second sequence of trial 1, when they received female urine swabs for the first time, followed by male stimulus animals, the response to female urine swabs was greater than it would have been if they had not received previous exposure to female urine. This is reflected in the difference between the total number of ultrasonic emissions from males (in the heterotypical group) exposed to female urine swabs for the first sequence and the total number of emissions from males exposed to female urine swabs for the second sequence. For the first sequence, the frequency of emissions was 4; for the second sequence, the frequency was 56. This explanation is also supported by the results of another analysis of variance performed on the ultrasonic responses to urine swabs during the acquisition phase of Experiment 2, dropping all responses to urine swabs on day 1 of acquisition.

In this analysis, the treatment X sex-urinary stimulus interaction was not significant ($F(1,18) = 3.44$; N.S.).

Extinction

Repeated presentations of female urine to a socially experienced male, in the absence of the female, cause the urine to cease eliciting ultrasounds. (It should be noted, however, that, if the acquisition phase had been continued, the heterotypical group's response to male and female urine swabs and the homotypical group's response to male and female urine swabs would probably have continued to decline). This ability of urine to lose its ultrasound eliciting potency is consistent with previous research findings on the biochemical elicitation of ultrasounds (Nyby et al., 1977; Dizinno et al., 1978). It seems that learning is centrally involved in the sexual responses to urine.

The significant difference between the heterotypical group's response to urine and the homotypical group's response to urine during the extinction phase was not expected. Further, it is not consistent with the acquisition phase in which no significant difference hetween the treatment groups' responses to urine was observed. Replication of this experiment would be necessary to determine whether or not the difference between the treatment groups in ultrasonic response to urine is the result of the treatment regimes.

Spontaneous Recovery

The attempt to demonstrate spontaneous recovery in Experiment 2 was not successful. One possible explanation is that the spacing of the conditioning trials over 8 days, with only 2 testing sequences per day, increased the probability of forgetting such that a recovery response would be highly improbable. This conditioning regime would be in contrast to amassing the trials into 1 or 2 days, for example, and then testing for spontaneous recovery. In the case of amassing the conditioning trials into 1 or 2 days, spontaneous recovery would be more likely to occur (Hilgard and Bower, 1975).

Pre- and Post-Tests with Animal Stimuli

Consistent with previous results in mouse chemocommunication research (Dizinno et al., 1978), naive males emitted ultrasounds to females upon first exposure, suggesting that some aspect of the female's phenotype acts as an unconditioned stimulus. However, the results of the post-tests for Experiment 1, and the results of the pre- and post-tests for Experiment 2, indicate that some discrimination of the gender characteristics of both sexes does occur. One interpretation of these results is that naive males are biologically predisposed to respond sexually to both male and female stimulus animals, but quickly learn to discriminate the males from the females.

Concluding Remarks

All of the above evidence on the biochemical elicitation of ultrasounds is consistent with the hypothesis that the acquisition of the "pheromonally" mediated behavior may be an instance of classical conditioning, where some aspect of the female's phenotype is the unconditioned stimulus and the female urine is the conditioned stimulus. Furthermore, the results of Experiments 1 and 2 are consistent with other mammalian chemical communication research demonstrating the dependency of many mammalian behaviors upon experience (Marr and Gardner, 1965; Mainardi et al., 1965; Carr et al., 1974; Porter and Etscorn, 1975; Porter and Doane, 1976).

The experiential component seems necessary for the male mouse ultrasonic response to female urine. Whether learning is a necessary component of other mammalian signalling "pheromone" systems in general remains an open question (Dizinno et al., 1978). However, my findings support the criticisms of some researchers that the concept of the classical "releaser" pheromone as applied to mammalian chemocommunication research is inappropriate (Bronson, 1974; Beauchamp et al., 1976).

The ultrasonic response of the male mouse to urine is a natural function, easily definable, and specific to the species, thus fulfilling two of the criteria variously used

for identifying a chemical substance as pheromonal (Bronson, 1974; Shorey, 1976). However, the sexual response of the male mouse to urine appears to be primarily determined by the prior experience of the animal and not by genetic factors. Further, urine is composed of hundreds of chemical substances (Bronson, 1976). These two characteristics of the mouse chemocommunication system do not fulfill two criteria variously used by researchers in identifying a chemical substance as pheromonal, namely, that the response be primarily genetically determined and the "pheromone" be composed of no more than a few compounds.

Bronson (1968) suggested that the category of "releaser" pheromone be amended to "signalling" pheromone. The signalling pheromone would provide information to the recipient which may or may not lead to a change in the recipient's behavior, depending upon its previous experience. Thus Bronson eliminated the criterion that the response be primarily genetically determined. If one or at most a few compounds could be isolated from mouse urine and be identified as the "carriers" of the signalling value of mouse urine, then the male mouse ultrasonic response to urine would fit Bronson's concept of the "signalling" pheromone system. However, previous research indicates that the male mouse ultrasonic response is not tightly constrained to urine; perfume will take on ultrasound

eliciting ability. These results suggest that all of the chemical components of urine that differentiate males from females may have signalling capabilities, and that the male mouse responds ultrasonically to all such components of urine.

If many chemical compounds in mouse urine are isolated and identified as having signalling capabilities, then Bronson's concept of the signalling pheromone as applied to mouse chemocommunication may not be appropriate. Further, the suggestion that the pheromone concept as applied to mammalian chemical communication be abandoned may be more appropriate (Beauchamp et al., 1976). The term "pheromone" would then refer only to a chemical compound which acted as a primer pheromone, releasing specific physiological responses. However, further research involving the isolation of chemical compounds in mouse urine and the identification of those compounds having signalling capabilities is necessary.

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APPENDIX - STATISTICAL ANALYSES

TABLE 1: ANOVA

EXPERIMENT 1: Urine Swab Test Phase

A = treatment
B = trials
C = sex-urinary stimulus
S = subjects

Source	ss	df	ms	F
A	197.43	1	197.43	18.13 (1,18)*
B	43.13	7	6.16	4.28 (7,126)*
C	8.39	1	8.39	2.64 (1,18) N.S.
AB	42.38	7	6.05	4.20 (7,126)*
AC	9.02	1	9.02	2.84 (1,18) N.S.
BC	5.72	7	.82	1.15 (7,126) N.S.
ABC	9.06	7	1.29	1.82 (7,126) N.S.
BS	181.48	126	1.44	-
CS	57.19	18	3.18	-
BCS	89.99	126	.71	-
S	196.10	18	10.89	-

*$p < .01$

TABLE 2: ANOVA

EXPERIMENT 1: Post-Test with Animal Stimuli

A = sex-animal stimulus
B = treatment group
S = subjects

Source	SS	df	ms	F
A	112.56	1	112.56	212.38*
B	16.26	1	16.26	2.97 N.S.
S(B)	43.74	8	5.47	-
AB	26.08	1	26.08	49.21*
AS(B)	14.97	28	.53	-

*$p < .01$

TABLE 3: ANOVA

EXPERIMENT 2: Urine Swab Test Phases

A = treatment
B = trials
C = sex-urinary stimulus
S = subjects

LEARNING PHASE

Source	ss	df	ms	F
A	30.55	1	30.55	1.92 (1,18) N.S.
B	176.78	7	25.25	11.12 (7,126)**
C	265.10	1	265.10	129.32 (1,18)**
AB	7.47	7	1.07	.47 (7,126) N.S.
AC	11.64	1	11.64	5.68 (1,18)*
BC	24.59	7	3.51	1.95 (7,126) N.S.
ABC	4.94	7	.71	.39 (7,126) N.S.
BS	285.89	126	2.27	-
CS	36.83	18	2.05	-
BCS	227.38	126	1.80	-
S	286.75	18	15.93	-

*p <.05 **p <.01

EXTINCTION PHASE

Source	ss	df	ms	F
A	101.37	1	101.37	4.57 (1,18)*
B	155.98	11	14.18	8.81 (11,198)**
C	401.98	1	401.98	77.30 (1,18)**
AB	16.90	11	1.54	.96 (11,198) N.S.
AC	3.18	1	3.18	.61 (1,18) N.S.
BC	39.96	11	39.96	24.37 (11,198)**
ABC	14.60	11	1.33	.81 (11,198) N.S.
BS	318.75	198	1.61	-
CS	93.60	18	5.20	-
BCS	325.27	198	1.64	-
S	399.11	18	22.17	-

*P <.05 **p <.01

TABLE 4: ANOVA

EXPERIMENT 2: Pre- and Post-Test with Animal Stimuli

A = sex-animal stimulus
B = treatment group
C = test #
S = subject

Source	ss	df	ms	F
A	206.76	1	206.76	75.46 (1,18)*
B	1.80	1	1.80	0.44 (1,18) N.S.
C	4.04	2	2.02	1.66 (2,36) N.S.
S(B)	74.31	18	4.13	-
AB	.78	1	.78	.28 (1,18) N.S.
AC	42.50	2	21.25	17.86 (2,36)*
BC	0.13	2	0.065	0.053 (2,36) N.S.
AS(B)	49.36	18	2.74	-
CS(B)	43.86	36	1.22	-
ABC	40.05	2	0.025	0.021 (2,36) N.S.
ACS(B)	42.85	36	1.19	-

* $p < .01$

VITA

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Professional Experience:

1980 (spring)	Instructor: "Introductory Psychology"
1979 (fall)	Paper Presentation: "Female Mouse Odors: The Role of Male Experience" Given to the Northeast Regional Meeting of the Animal Behavior Society. Marine Biological Laboratory, Woods Hole, Mass. Research supported by National Science Foundation grant.
1978-present	Associate Staff for Inter-Varsity Christian Fellowship. Involves training college students and speaking to Inter-Varsity student chapters.
1978-present	Research Assistant in the psychology department at Lehigh University.
1978-present	Teacher of Bible Study and Fellowship group at First Presbyterian Church, Beth., Pa.
1977-1978	Teaching Assistant for the psychology department at Lehigh University.
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