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ULTRASONIC COMMUNICATION AND THE HORMONAL MODULATION OF AGGRESSIVE BEHAVIOR IN THE FEMALE HAMSTER

A thesis submitted to the Faculty of The Rockefeller University

in partial fulfillment of the requirements

for the degree of Doctor of Philosophy

by

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PREFACE

It is impossible to adequately acknowledge the varied contributions to this work of my colleagues, friends and family. Special thanks, however, go to my advisors, Drs. Donald Pfaff and Carl Pfaffmann, for their advice, encouragement and support.

ABSTRACT

Female golden hamsters are unusual both in the high levels of aggression they exhibit on nonestrous days of the estrous cycle and in the dramatic decrease in aggressiveness apparent on estrous days. The results of studies using adrenalectomized-ovariectomized or hypophysectomized females have failed to reveal any individual ovarian or pituitary hormone that is required for the display of high levels of aggression. In contrast, it seems clear that a very specific combination of ovarian hormones normally accounts for the inhibition of fighting and stimulation of sexual receptivity seen on estrous days. Thus, while control adrenalectomized-ovariectomized females fought at high levels comparable to those seen in intact nonestrous females, treatment with a combination of 17β-estradiol benzoate and progesterone suppressed fighting completely, creating a situation identical to that typical of estrous day.

The aggressiveness of male and female hamsters suggests the operation of a social system in which individuals live solitarily and depend on noncontact social signals for the initiation and coordination of reproductive behavior. In this regard, the studies detailed here show that hamsters of both sexes emit ultrasonic calls of 16-64 kHz and roughly 50 dB SPL. Ultrasounds by females encode information regarding reproductive state as well as location. In particular, female call rates are highest during estrus, suggesting that these signals function primarily as sexual attractants in the initiation of social contact. Once contact has been initiated, ultrasounds by males prolong lordosis, thus helping to structure the interaction so as to facilitate successful reproduction. As in the case of the aggressive responses described above, hamster ultrasounds seem well suited to a behavioral system that strives to coordinate crucial reproductive behaviors with reproductive (endocrine) state.

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CHAPTER I. STEROID HORMONES AND AGGRESSION IN NONHUMAN VERTEBRATES

ANDROGENS AND AGGRESSIVE BEHAVIOR OF MALES

In a variety of species, the aggressiveness of a male is related closely to his reproductive condition (Guhl, 1961). Such correlations have proved most conspicuous in developing individuals and among seasonal breeders. For example, in young male mice (Mus musculus), isolated except during brief daily encounters, the appearance of fighting at 34-36 days of age seems to coincide with the attainment of sexual maturity (Fredericson, 1950; Kirkham, 1920).

In seasonally breeding species, the reproductive phase of the annual cycle may be associated with dramatic alterations in social organization (Guhl, 1961). Increases in the frequency of male-male antagonism often may be instrumental in the establishment and defense of breeding territories. For example, gradual increases in the frequency of antagonistic interactions between individuals of the same sex accompanies the breeding season in free-ranging ring-necked pheasants (Phasianus colchicus) (Collias & Taber, 1951). Frequent aggressive episodes among males seem to represent instances of territorial defense and may be related to a seasonal increase in testes weight.

Field studies of animal social behavior also have focused attention on correlations between the dominance rank or aggressiveness of individual males and their reproductive success. Among free-ranging rhesus monkeys (Macaca mulatta), the sexual activity of individual males is related directly to their dominance ranks (Conaway & Koford, 1965; Kaufmann, 1965). Similarly, variation in copulation frequency within a wild population of tassel-eared squirrels (Sciurus aberti ferreus) was closely tied to frequencies of participation and success in aggressive encounters (Farentinos, 1972). The first male to copulate during a mating bout was invariably the dominant male in agonistic interactions occurring during that bout.

The studies summarized above suggest that the tendency of a male to engage in aggressive interactions with male conspecifics may be related to gonadal activity. A more direct approach to the investigation

of correlations between gonadal activity and aggressivity has been used by Rose, Holaday and Bernstein (1971). Here, plasma testosterone concentrations of male rhesus monkeys have been measured directly and found to correlate positively with both dominance rank and frequency of aggressive behavior. At least in the case of dominance rank, however, the significant correlation with testosterone levels seems to be attributable largely to the relatively few animals occupying extremely high positions within the hierarchy. Thus, males in the highest quartile with respect to dominance rank had significantly higher concentrations of plasma testosterone than did less dominant animals. No significant differences in testosterone levels were observed among males in any of the lower three quartiles.

While strongly suggestive of a causal relationship between androgens and aggressive behavior, the above studies are limited to demonstrating correlations between some indices of male gonadal activity and dominance rank or frequency of participation in agonistic interactions. Such correlations might have arisen from a direct dependence of sexual and aggressive activities on each other, or from their mutual dependence on some third factor. More complete evidence establishing a direct dependence of aggression on gonadal androgens requires the demonstration that castration produces a decrease in the frequency of aggressive behaviors and that this change is reversed by androgen replacement therapy.

At least some strains of mice satisfy these criteria for the androgen-dependence of aggressive behavior (e.g., Beeman, 1947; Edwards, 1969: Sigg, 1969; Suchowsky, Pegrassi & Bonsignori, 1969). Beeman (1947) staged extensive series of round-robin encounters among inexperienced C57 black mice castrated at least 25 days prior to the beginning of testing. Twenty-five mice segregated among 7 groups of 3-4 individuals exhibited no attacks or fights in a total of 198 encounters. Following the implantation of testosterone propionate (TP) pellets (estimated to be absorbed at an average rate of 0.15 mg per day), a subset of the original group consisting of 14 males exhibited a total of 391 attacks and 234 fights in 108 encounters, levels of aggression comparable to those displayed by intact males. The complete removal of androgen pellets from a single group of 3 mice

resulted in a profound decrease in the frequency of aggressive behaviors. While this group had compiled 48 attacks and 18 fights in the 36 encounters during testosterone treatment, only 3 attacks appeared in the same number of encounters following the removal of the hormone pellets. Clearly, the castration of male mice typically is accompanied by a decrement in aggressive behavior. This decrease may be partially or totally reversed by treatment with TP.

Like castration, hypophysectomy prevents the development of fighting behavior in male mice. This decrement, too, may be at least partially alleviated by testosterone replacement therapy (Sigg, 1969). This suggests that the induction of fighting in castrated male mice by testosterone involves direct effects of androgens on neural mechanisms mediating aggression in the male.

Other species in which androgens have been implicated in the control of male-male aggressive behavior include the following: the gobiid fish, Bathygobius soporator (Tavolga, 1955); the lizard, Sceloporus grammicus microleptidotus (Evans, 1946); domestic fowl, Gallus domesticus (Domm, 1939); Japanese quail, Coturnix coturnix japonica (Selinger & Bermant, 1967); red grouse, Lagopus lagopus scoticus (Watson, 1970); ring doves, Streptopelia risoria (Bennett, 1940; Erickson, Bruder, Komisaruk & Lehrman, 1967); valley quail, Lophortyx californica vallicola (Emlen & Lorenz, 1942); weaver birds, Quelea quelea quelea, Ploceinae (Crook & Butterfield, 1968); the golden hamster, Mesocricetus auratus (Payne & Swanson, 1972a and 1972b; Vandenbergh, 1971); the domestic laboratory rat, Rattus norvegicus (Barfield, Busch & Wallen, 1972); Mongolian gerbils, Meriones unguiculatus (Sayler, 1970); red deer, Cervus elaphus (Lincoln, Youngson & Short, 1970; Lincoln, Guinness & Short, 1972); roe deer, Capreolus capreolus (Bramley, 1970); and, a chimpanzee, Pan troglodytes (Clark & Birch, 1945). Inclusion in this list requires the demonstration that castration reduces the incidence of aggression in adult males or that androgen treatment of intact or castrated adults occasions an increase in the incidence of fighting or advancement within a dominance hierarchy. Factors limiting the hormonal-dependence of aggression in some of these species are discussed below.

In several studies listed above, individuals subjected to androgen therapy were selected on the basis of low dominance rank or inability to defend a territory. Such individuals may have low levels of endogenous gonadal steroids (see Rose et al., 1971). Sexually immature males represent an analogous case. Androgen injections or implants have induced the early appearance of fighting behavior or dominance relations among immature males of a variety of species: the lizard, Anolis carolinensis (Noble & Greenberg, 1941); domestic fowl (Collias, 1950; Guhl, 1958; Noble & Zitrin, 1942); herring gulls, Larus argentatus (Boss, 1943); and, the laboratory mouse (Levy & King, 1953). In the last species, the neonatal castration of males results in a decrement in intermale aggressive behavior unless exogenous testosterone is administered within the few days immediately following birth (Bronson & Desjardins, 1969; Edwards, 1969; Peters, Bronson & Whitsett, 1972).

Recent experiments have attempted to specify the chemical forms of androgen responsible for hormonally-induced aggression. For example, the naturally occurring free alcohol form of testosterone effectively induces fighting behavior in castrated male mice confronted with relatively non-aggressive opponents (group-housed male castrates) (Luttge, 1972). Androstenedione, the metabolically oxidized product of testosterone, was ineffective in the elicitation of intermale aggression under the same circumstances. However, castrated male mice confronted with intact, isolated (and presumably more aggressive) male opponents were responsive to the same androgen (Erpino & Chappelle, 1971). Here, the implantation of 30 mg pellets of androstenedione was associated with attack latencies which were significantly lower than those exhibited by cholesterol-implanted controls, and, in fact, were comparable to those associated with TP treatment. Androsterone, dehydroisoandrosterone and dihydrotestosterone are ineffective at inducing aggressive behavior in castrated male mice (Erpino & Chappelle, 1971; Luttge, 1972). Orally administered methyl testosterone is similarly ineffective in doses of up to 0.8 mg per day (Bevan, Bevan & Williams, 1958). Finally, administration of the anti-androgenic steroids cyproterone or cyproterone acetate to TP-treated castrate mice

(Edwards, 1970a) or to intact gerbils (Sayler, 1970) failed to induce a decrease in the incidence of aggressive behavior, though both anti-androgens exerted significant effects on androgen-sensitive peripheral tissues.

Pecking and bow-cooing directed at stimulus males have been elicited in castrated male ring doves by TP-implants in the anterior hypothalamic-preoptic area of the brain (Barfield, 1971). Similarly, some elements of aggressive behavior were exhibited by castrated male domestic fowl receiving TP implants in the lateral forebrain (Barfield, 1965). TP implants in the preoptic area of capons elicit copulatory behavior, but not courtship or aggressive behaviors (Barfield, 1969).

Studies summarized above include many relatively unambiguous demonstrations of the androgen-dependence of aggressive behavior in a variety of species. In contrast to this array of consistent results, some other studies have failed to convincingly implicate androgens in the mediation of aggressive behavior. Male starlings and weaver birds provide particularly striking examples in that a specific hormone (LH) other than the gonadal androgens seems to underlie agonistic encounters not associated with competition for nest-building materials (Crook & Butterfield, 1968; Davis, 1957; Davis, 1964; Mathewson, 1961). Further negative findings regarding the androgen-dependence of aggression have been reported under some conditions for the males of a variety of other species: swordtail fish, Xiphophorus helleri (Noble & Borne, 1940); the lizard, Anolis carolinensis (Evans, 1936; Greenberg & Noble, 1944; Noble & Greenberg, 1941); black-crowned night herons, Nycticorax nycticorax hoactli (Noble & Wurm, 1940); pigeons, Columba livia (Carpenter, 1958; Lumia, 1972); ring doves (Vowles & Harwood, 1966); dogs, Canis familiaris (Le Boeuf, 1970); the golden hamster (Tiefer, 1970); the laboratory mouse (Bevan et al., 1958; Bronson & Desjardins, 1969; Burge & Edwards, 1971; Edwards, 1969; Suchowsky et al., 1969; Uhrich, 1938); Mongolian gerbils (Anisko, Christenson & Buehler, 1973); the laboratory rat (Conner & Levine, 1969; Conner, Levine, Werthein & Cummer, 1969); roe deer (Bramley, 1970); squirrel monkeys, Saimiri sciureus (Green, Whalen, Rutley & Battie, 1972); and, rhesus monkeys, Macaca mulatta (Mirsky, 1955).

FACTORS AFFECTING HORMONE RESPONSIVENESS

Differences among some of the results summarized above are most troublesome if aggression is considered as a monolithic behavior type, a viewpoint which certainly would be fallacious. Distinct categories of aggressive behavior have been defined according to the stimulus situation provoking destructive attack (Moyer, 1968). Different categories of aggressive behavior may be mediated by different physiological substrates. Thus, hormonal states which modulate aggressive responses in one stimulus context may have little effect when the stimulus situation is altered. For example, TP-treated male ring doves, housed in isolation or in male-female pairs, failed to differ from controls with respect to the incidence of aggressive behavior evoked by hand-held conspecifics (Vowles & Harwood, 1966). In contrast, in group-caged unisexual flocks of doves, the administration of TP to low-ranking, submissive, individuals resulted in an increased display of aggression and advancement within the social hierarchy (Bennett, 1940).

These results emphasize the complexity and context-dependence of agonistic behaviors. Factors which must be considered in the interpretation of aggressive behavior include the following: (a) possible functions of aggressive behavior in relation to the natural social organization and ecology of the species; (b) characteristics of the opponent; (c) the species and strain of animal studied; (d) the prior experience and reinforcement history of the individual; and, (e) the system of measurement employed to monitor variations in aggressiveness.

(a) Intraspecific aggressiveness may facilitate the distribution of vital resources among the members of a community. Crook and Butterfield (1968) have suggested that the degree to which a particular commodity is required for successful reproduction is related to the hormonal basis of competition for that commodity: "Hierarchies established in relation to competition for sexually relevant objectives are likely to be influenced by androgen whereas those in relation to quarrels without sexual significance will not be so affected" (p. 383).

Accordingly, TP injections do not affect the relative dominance of male weaver birds in agonistic encounters stemming from individual distance infringements, but they do increase success in competitive encounters occasioned by the presence of nest-building materials.

(b) Opponents differing in size, age or hormonal condition may provoke distinct reactions on the part of an individual. Tested in within-treatment pairs in a neutral arena, female mice are quite non-aggressive unless treated neonatally with androgens (e.g., Edwards, 1968; Edwards, 1969). Moreover, unless neonatally androgenized, aggression among adult females is not readily modified by exogenous TP treatment in adulthood (Bronson & Desjardins, 1970; Edwards, 1969; Edwards & Herndon, 1970; Levy, 1954). However, an ovariectomized female tested in her home cage against a prepuberal male exhibits significant levels of aggressive behavior, and the aggressiveness of such a female increases with adult TP, but appears to be un-responsive to neonatal TP (Edwards, 1969). Thus, in several important respects, changing the age/sex class of the opponent and other details of the testing situation reversed the results.

Regardless of hormonal condition, fighting may be observed in a normally non-aggressive animal if provoked sufficiently by a more aggressive opponent. Thus, castrated male rats encountering intact opponents in a neutral area exhibited low levels of several agonistic behaviors (Barfield et al., 1972). Fighting within such pairs was extremely infrequent. Nevertheless, on the rare occasions that violent fighting did occur, castrates fought successfully and were not consistently submissive to their intact opponents. These results are reminiscent of the emphasis laid by Scott and Fredericson (1951) upon the importance of pain as a primary releaser of aggressive behavior.

(c) Different species, and different strains within a single species, differ in their sensitivity to hormone treatment. For instance, though within-treatment pairs of castrate male mice fail to fight in the absence of exogenous TP (e.g., Beeman, 1947), prepuberally castrated male dogs competed as intensely among themselves for access to a receptive female as did a group of intact controls (Le Boeuf, 1970).

Among different strains of mice, the ability of neonatal TP treatment to induce aggression in females varies significantly and may be related to the aggressiveness exhibited by males of the same strain (Vale, Ray & Vale, 1972). The aggressive behavior of neonatally androgenized females was increased significantly only among females from inbred strains in which normal males exhibited high levels of aggression. Thus, the masculinization of females with respect to aggression apparently requires the activation of mechanisms which are shared with males of the same strain, but which are normally inactive in females due to low levels of endogenous androgen during a critical developmental period.

(d) The prior experience and reinforcement history of an individual in agonistic interactions often may be a more potent determinant of aggressiveness than hormonal state. Mice engaged in a competitive task motivated by aversive stimulation (foot shock) performed largely in accordance with the type of pre-test training experienced (Bevan, Daves & Levy, 1960). Individuals trained as "winners" exhibited longer and more intense bouts of fighting than individuals trained as "losers" or individuals receiving no prior training. While androgen status also affected competitive fighting, this seemed to represent an indirect effect exerted by virtue of a positive relationship between androgen treatment and body weight. Similarly, while spontaneous, or isolation-induced, fighting among inexperienced adult male mice requires endogenous or exogenous androgens (e.g., Beeman, 1947), highly experienced male castrates confronted with a relatively nonaggressive opponent may continue to fight for at least eight weeks following gonadectomy (Burge & Edwards, 1971).

Social relationships among laboratory mice may be quite rigid. Having achieved dominance over a conspecific, a male mouse may maintain his high social rank despite severe debilitation induced by vitamin B_1 deficiency (Beeman & Allee, 1945). In more natural situations, the rigidity of social hierarchies in some species may be due to the differing reinforcement histories of individual group members. While the aggressiveness of free-ranging male valley quail was increased by androgen administration, no reversals in dominance rank resulted from hormone treatment of submissive individuals (Emlen & Lorenz, 1942).

(e) Finally, inconsistent results concerning the effects of hormones on aggressive behavior may stem from idiosyncratic or ambiguous systems of measurement. Behavioral measures sometimes are described incompletely or in subjective terms. Even though the sensitivity and relevance of behavioral elements as measures of aggressiveness may vary with their placement in the agonistic sequence, measures sometimes are lumped for the purposes of brevity and statistical analysis (e.g., Payne & Swanson, 1970). The presentation of a variety of distinct measures corresponding to different intensities of aggressive behavior seems to represent a more informative approach.

The above list is far from an exhaustive compilation of the many variables that probably modulate the hormonal dependence of aggressive behavior. Nevertheless, it is clear that valid generalizations about effects of androgens, and other hormones, on aggression will have to take into account the natural structure and context of the behavior considered, as well as factors such as the species and condition of the aggressive animal and its opponent. These reservations apply even when emphasis has been restricted, as in this review, to studies of intraspecific fighting occurring "spontaneously" or as a consequence of isolation or the limited availability of specific commodities.

EFFECTS OF PROGESTERONE AND ESTROGENS ON AGGRESSION IN MALES

Progesterone (P) administration to intact male mice produces a significant decrease in the probability of fighting (Suchowsky et al., 1969). Similarly, in castrated male mice, the combinations of P and either TP or androstenedione are associated with lower frequencies of aggressive behavior than those evoked by either androgen alone (Erpino & Chappelle, 1971; Luttge, 1972). Treatment of castrated male mice with P alone results either in no significant effect on the incidence of aggression (Erpino & Chappelle, 1971; Luttge, 1972), or, in a moderate, but possibly transient, induction of fighting (Suchowsky et al., 1969).

P can affect androgen-dependent aggressive behavior in male mice without interfering with the maintenance of androgen-dependent peripheral tissues (Erpino & Chappelle, 1971; Luttge, 1972). This

distinction, and the ability of high doses of TP to reverse P-induced suppression of fighting (Luttge, 1972) suggests the occurrence of a direct competitive antagonism between P and androgens at the level of the central nervous system (CNS). Consistent with this notion is the observation of reduced uptake of tritiated testosterone by the rat brain as a consequence of P pretreatment (Stern & Eisenfeld, 1971).

Effects of P on bow-cooing in male ring doves (a behavior appearing in both aggressive and sexual contexts) parallel many of those on male-male fighting in mice. Systemic P administration suppresses bow-cooing in intact males or TP-treated castrates (Erickson et al., 1967). P alone does not induce bow-cooing in castrated male doves. Moreover, the suppression of bow-cooing in intact male doves during heterosexual pairings was associated with P implants in the anterior hypothalamus, preoptic nuclei and the lateral forebrain system (Komisaruk, 1967). These neuroanatomical loci are similar to those at which TP implants elicited pecking and bow-cooing in castrated male doves during male-male encounters (Barfield, 1971).

Effects of progestins on male aggression depend upon the species and conditions of testing. A particularly important consideration may be the age/sex class of the opponent. Progesterone treatment of castrated male hamsters results in enhanced success in encounters with intact non-receptive females (Payne & Swanson, 1972b; see also, Payne & Swanson, 1971a).

Estrogens have been associated with suppressive effects upon aggressive behavior. In intact male individuals representing a variety of species, estrogens either have suppressed the incidence or vigor of aggressive behavior (immature domestic fowl, Guhl, 1958; red grouse, Watson, 1970; mice, Banerjee, 1971; Bronson & Desjardins, 1968; Suchowsky et al., 1969; Terdiman & Levy, 1954; and, rats, Work & Rogers, 1972) or have had no significant effect (ring doves, Vowles & Harwood, 1966; an immature black-crowned night heron, Noble & Wurm, 1940; valley quail, Emlen & Lorenz, 1942; mice, Gustafson & Winokur, 1960; and, young rhesus monkeys, Mirsky, 1955). Inhibitory effects of estrogens on the aggressivity of intact males could represent indirect effects involving interference with the production or activity

of testicular androgens. Neonatal injections of estradiol benzoate (EB) result in decreased body weights, decreased relative testes and seminal vesicle weights and a decrement in the amount of fructose in the seminal vesicles of intact male mice (Bronson & Desjardins, 1968). The 72 percent reduction in seminal vesicle fructose (a correlate of androgen titers) suggests that the decreased incidence of fighting among neonatally treated males stemmed from insufficient circulating levels of endogenous androgens, rather than from a direct effect of estradiol upon neural mechanisms involved in the mediation of aggressive behavior. Intact individuals may constitute inappropriate subjects for the investigation of exclusively CNS-mediated effects of exogenous estrogens on male aggressive behavior.

The administration of exogenous estrogens resulted in suppression of the aggressive behavior of castrated male herring gulls (Boss, 1943) and chimpanzees (Clark & Birch, 1945). The only clear instance of suppression described in the former study concerned a single male castrate receiving both estradiol dipropionate and TP. Clark and Birch (1945) describe a single, prepuberally castrated male chimpanzee which exhibited lower levels of success in food competition with an intact partner during periods of treatment with alpha estradiol. However, the interpretation of changes in dominance status occurring during hormone treatment is complicated by variable results, and by concurrent changes in test location, known to have dramatic effects upon the social relations of the two males.

Recent studies of aggressive behavior in castrated male hamsters indicate that ovarian implants or exogenous EB facilitate intermale fighting (Payne & Swanson, 1971a and 1972b; Vandenbergh, 1971). Untreated male castrates typically exhibit less aggression than, and are submissive to, their intact male opponents. Treatment with as little as 10-25 µg per day of EB results in elevated frequencies of several agonistic behaviors and in increased relative dominance by the castrates. In this regard, EB replacement therapy seems to be at least as effective as is treatment with TP (Vandenbergh, 1971). Among Swiss-Webster albino mice, treatment of castrated males with EB facilitates the display of aggressive behavior in encounters with untreated male

castrates (Edwards & Burge, 1971). In contrast, despite similar opponents and test situations, castrated males of the CD-1 strain seem to be unaffected by EB replacement therapy even though TP did facilitate intermale fighting (Luttge, 1972).

Administration of exogenous TP or EB to castrated male hamsters proves ineffective at inducing relative dominance over intact females (Payne & Swanson, 1972b). This is consistent with the dominance normally exhibited by intact females over intact males (e.g., Payne & Swanson, 1970).

In summary, if estrogens affect the aggressive behavior of castrated males at all, they operate to increase the incidence of intermale fighting. Thus, in aggressive behavior, as in the case of at least some aspects of sexual behavior (Edwards & Burge, 1971; Davidson, 1969; Pfaff, 1970a) estrogens may mimic behavioral effects of androgens. In each case, direct effects of estrogens upon central neural mechanisms mediating the behavior in question are presumed.

At least in some strains of mice, pituitary ACTH and/or adrenal steroids under the control of ACTH are capable of modulating aggressive behavior (Brain, 1972a; Brain, Nowell & Wouters, 1971; Leshner, 1972; Sigg, 1969; see also, Burge & Edwards, 1971). Particularly in early encounters between inexperienced adult males, adrenalectomy or ACTH-treatment result in a partial suppression of fighting behavior (Brain et al., 1971; Harding & Leshner, 1972; Leshner, 1972; Leshner, Walker, Johnson, Kelling, Kreisler & Svare, 1973; Sigg, 1969). Suppressive effects of adrenalectomy probably stem from increased endogenous ACTH levels, occasioned by the elimination of adrenal glucocorticoids which normally inhibit ACTH release. Accordingly, treatment with corticosterone, dexamethasone, or hydrocortisone is associated with increased levels of isolation-induced aggression (Brain et al., 1971; Kostowski, Rewerski & Piechocki, 1970; Leshner, 1972; Leshner et al., 1973; Sigg, 1969). In contrast, desoxycorticosterone, an adrenal mineralocorticoid, does not affect the latency or vigor of isolation-induced fighting in mice (Kostowski et al., 1970). Various combinations of adrenal and

gonadal manipulations suggest that the adrenals and testes constitute independent systems modulating levels of inter-male aggression in mice (Leshner, 1972; Leshner et al., 1973).

HORMONES AND AGGRESSION IN FEMALES

The fighting behavior of many species is sexually dimorphic, females generally exhibiting lower frequencies of aggressive behaviors than males (Collias, 1944; Guhl, 1961). Direct comparisons of ovariectomized and intact females have yielded variable results. Six members of a flock of 12 hens decreased in social rank following ovariectomy (Collias, 1944); no ovariectomized hen occupied a hierarchical position above that of the least aggressive intact individual. Nevertheless, the pre-operative ranks of the six operated birds with respect to each other survived gonadectomy, suggesting that not all manifestations of aggressivity were eliminated by ovariectomy. Female swordtails maintained their pre-operative social ranks for 1 - 3 months following gonadectomy (Noble & Borne, 1940). Similarly, frequencies of aggressive responses exhibited by female rhesus monkeys in male-female encounters failed to vary significantly as a result of ovariectomy (Michael & Zumpe, 1970). In contrast, female lizards (Anolis carolinensis) exhibited elevated levels of aggression as a consequence of gonadectomy (Evans, 1936). Ovariectomy of female weaver birds during the breeding season, but not at other times, is associated with an increase in female-female aggressive encounter frequency (Lazarus & Crook, 1973). Among female mice, too, a slightly increased incidence of fighting may accompany castration (Suchowsky, Pegrassi & Bonsignori, 1971).

The aggressive behavior of adult female mice is relatively insensitive to treatment with TP or EB (Bronson & Desjardins, 1970; Edwards, 1968; Edwards, 1969; Edwards, 1970b; Edwards & Burge, 1971). While fighting occurs in all male-male pairs in which both members have been gonadectomized at 30 days and treated with TP as adults, only 8% of similarly treated female-female pairs exhibit fighting (Edwards, 1968). The insensitivity of females to adult TP depends, in part, upon the age/sex characteristics of the opponent. Ovariectomized

females tested in their home cages against prepuberal male opponents exhibit significant levels of aggression in the complete absence of hormone replacement, and are responsive to adult TP treatment (Edwards, 1969).

Females of a variety of species have exhibited elevated levels of aggressive behavior during adult treatment with androgens: the lizard, Sceloporus grammicus (Evans, 1946); canaries (Shoemaker, 1939); chickens (Allee, Collias & Lutherman, 1939; Davis & Domm, 1943; Douglis, 1948; Guhl, 1958; Williams & McGibbon, 1956); herring gulls (Boss, 1943); immature black-crowned night herons (Noble & Wurm, 1940); ring doves (Bennett, 1940); and, chimpanzees (Birch & Clark, 1946). An increased incidence of aggression among female swordtail fish subjected to prolonged androgen therapy seemed to be incidental to a more complete sex reversal (Noble & Borne, 1940). Only within a group of three ovariectomized chimpanzees did the facilitatory effect of TP seem to be duplicated by EB (Birch & Clark, 1946). In several other species, EB-treatment of adult or immature individuals appears to have little or no effect on aggressivity: swordtails (Noble & Borne, 1940); canaries (Shoemaker, 1939); chickens (Allee & Collias, 1940; Guhl, 1958); and, night herons (Noble & Wurm, 1940).

In contrast to results summarized above, the aggressive behavior of intact adult female valley quail is insensitive to treatment with either TP or EB (Emlen & Lorenz, 1942). Similarly, no changes in dominance rank within groups of immature female rhesus monkeys occurred as a consequence of TP- or EB-therapy (Mirsky, 1955). However, at least some classes of aggressive responses exhibited by ovariectomized adult rhesus monkeys tested in male-female pairs may be subject to modification by EB (Michael & Zumpe, 1970). While the overall level of aggression exhibited by untreated ovariectomized females persisted during EB treatment, aggressive responses which were not associated with mounting attempts by the male partner appeared to increase during hormone therapy.

Androgen treatment of ovariectomized Mongolian gerbils resulted in a significant decline in aggression compared with gonadectomized females receiving only control injections (Anisko et al., 1973).

Among ovariectomized female weaver birds, estrogen therapy decreased aggressive encounter frequency, while agonistic behaviors occurring as a result of individual-distance infringements were facilitated by pituitary LH (Lazarus & Crook, 1973).

Sexual dimorphisms in the spontaneous aggressivity of male and female mice, as well as in the responsiveness of gonadectomized individuals to adult TP, have been attributed to a sexually differentiated neural substrate ordinarily "organized" in the genetic male by (endogenous) neonatal androgens (e.g., Edwards, 1968). The ability of neonatally administered TP to facilitate androgen-aroused fighting in adult gonadectomized female mice provides strong support for this interpretation (Bronson & Desjardins, 1970; Edwards, 1968; Edwards, 1969; Edwards, 1970b; Edwards, 1971; Edwards & Herndon, 1970; Whitsett, Bronson, Peters & Hamilton, 1972). For example, fighting occurred in 95% of the pairs of ovariectomized female mice treated neonatally with 0.5 mg TP and tested as adults under increasing doses of TP (Edwards, 1968). In contrast, only 20% of pairs treated with oil neonatally fought at any dosage of adult TP. Neonatal effects of TP upon later androgen-aroused fighting may be mimicked by neonatally administered EB (Edwards & Herndon, 1970) or testosterone, but not by androstenedione or the combination of testosterone and androstenedione (Edwards, 1971).

The effectiveness with which exogenous androgen is able to "masculinize" the female CNS with respect to aggressive behavior depends upon the timing and extent of early hormone treatment. The efficacy of a given dosage of TP seems to be related directly to its temporal proximity to birth (Bronson & Desjardins, 1970; Edwards, 1969; Edwards, 1970b; Whitsett et al., 1972). Thus, 80% of cages, each housing four females which had been treated with 0.4 mg of TP within 2 hours after birth, contained at least one wounded individual (Whitsett et al., 1972). In contrast, females receiving the same dose of TP 48 $\pm$ 1 hour postbirth failed to engage in fighting severe enough to produce wounding. More sensitive indices of aggression demonstrate that single injections of TP (0.4 mg/g body weight) may significantly affect aggressive behavior in females treated as late as 12 days following birth (Bronson & Desjardins, 1970; see also Edwards, 1969). In fact, females treated

with 0.1 mg TP per day for 20 days beginning on the thirtieth day following birth exhibited significantly more androgen-induced fighting than females treated with oil according to the same schedule (Edwards, 1970b). While a "critical period" for androgenization may exist for any particular regime of neonatal TP treatment, the limits of this period are flexible. Different regimes of neonatal hormone treatment and different measures of aggression in adulthood are associated with different "critical periods."

The sexual dimorphism discussed above depends, to some extent, on the situations in which aggressive behavior is observed. Normally, nonaggressive female mice exhibit immediate and intense aggression toward male or female conspecifics during lactation (Gandelman, 1972; Noirot, 1968; Svare & Gandelman, 1973). The experimental analysis of the hormonal basis for this dramatic increase in female aggressiveness has not yet been reported.

CHAPTER II. DESCRIPTION OF FEMALE HAMSTER AGGRESSION

Unlike the females of many vertebrate species, female golden hamsters (Mesocricetus auratus Waterhouse) are highly aggressive at most times and tend to dominate males in heterosexual pairings (Dieterlen, 1959; Payne & Swanson, 1970 and 1972c). This contrast has stimulated much interest in the hormonal basis for female hamster aggressivity. Nevertheless, reports conflict regarding the effects of various hormonal manipulations. For example, ovariectomy has been associated with a slightly decreased incidence of female aggression in heterosexual (Kislak & Beach, 1955; Tiefer, 1970; Payne & Swanson, 1971b and 1972c) and unisexual (Payne & Swanson, 1971c) pairings. On the other hand, Vandenbergh (1971) has been unable to distinguish between levels of aggression exhibited by ovariectomized and intact individuals in female-female encounters.

Contradictory reports regarding the effects of ovariectomy, and other hormonal manipulations, on female hamster aggressivity may stem, in part, from drastic differences in the amount of social contact experienced by subjects before and during experimental encounters. For example, most previous studies of female hamster aggression have employed group-caged females (Payne & Swanson, 1970, 1971b-c and 1972c). Further, experimental aggressive encounters have been frequent and prolonged, sometimes including as many as 4-5 hours of social contact with strange conspecifics (Kislak & Beach, 1955; Payne & Swanson, 1971b). Such conditions may occasion very complex social interactions, differing in many respects from those typical of earlier encounters among less socially-experienced individuals. In particular, susceptibility to physiological manipulation may depend upon previous social experience. Dramatic increases in the aggressiveness of socially naive and socially experienced dominant male hamsters followed septal ablation (Sodetz & Bunnell, 1970). However, septal hamsters which had experienced defeat during preoperative testing failed to display any increment in aggressiveness.

Valid generalizations regarding relations among hormones and aggressive behavior may have to reflect the natural structure and context of social interactions in the particular species considered:

"Hierarchies of social status are...not to be conceived solely in relation to encounters between individuals, but in the context of the function of aggressive behaviour in relation to the environmental norms of the species" (Crook & Butterfield, 1968, p. 383). Accordingly, we may inquire as to the extent to which the group-housing of subjects and their participation in relatively long encounters in confined arenas mimic the natural structure and context of hamster social interactions. So far, direct observations of the behavior and ecology of wild hamsters have been insufficient to resolve this question (Murphy, 1971). Nevertheless, the relatively invariant high level of aggressiveness characteristic of male and female hamsters suggests strongly that free-ranging individuals of this species lead a relatively solitary existence. Encounters between conspecifics might be rare and fleeting, providing no natural analogues corresponding to the group-housed individual or the extended social interactions to which he often is subjected. These considerations suggest that the initial phases of a social interaction, those preceding persistent escape attempts by either participant, might be of critical importance in hamster social relations. Thus, the early stages of an encounter series might provide the most interesting setting in which to investigate the hormonal, and other physiological, bases for female hamster aggressive behavior.

An accurate and detailed description of hamster agonistic postures and behavior sequences is essential to the evaluation of physiological effects. Previous studies of hamster aggression (e.g., Grant & Mackintosh, 1963; Bunnell, Sodetz & Shalloway, 1970; Payne & Swanson, 1971c and 1972c; Lerwill & Makings, 1971) have employed quantitative measures of aggression which stress differences between stereotyped "offensive" and "defensive" postures. However, we feel that, among hamsters, the differentiation of "offensive" and "defensive" postures may be possible only at advanced stages of an interaction, following the establishment of a highly polar dominance-subordination relationship. Thus, these postures, like the rigid social relations that occasion them, may be artifacts of experimental confinement. During encounters among young male hamsters, most "full aggressive" and "full submissive" postures followed an initial attempt to escape by one participant

(Lerwill & Makings, 1971). In more natural situations, flight might effectively terminate any such social interaction, rendering ritualized threat and appeasement unnecessary. In more gregarious species distinctive submissive postures might be necessary for the retention of group membership by a low-ranking individual. Here, prolonged confinement to an experimental arena may have some analogue in the cohesiveness of the social group. It is questionable, however, whether such a social organization, or any such analogue exists for hamsters. Accordingly, strong emphasis on the recognition of stereotyped offensive or defensive postures may be inappropriate in selecting experimental measures of hamster aggressiveness. At least during the initial encounters in a series of aggression tests, hamster agonistic postures do not seem to be highly stereotyped or ritualized. Rather, individual agonistic postures exhibit considerable variability in form, and relatively extreme forms (such as might be thought to represent "offensive" and "defensive" subdivisions of the posture in question) may be exhibited by both participants in an encounter in rapid alternation (unpublished observations). However, while individual postures adopted by female hamsters are not highly ritualized, we have found that the sequence in which qualitatively distinct postures, or behaviors, appear during an encounter between relatively inexperienced nonestrous females is orderly and somewhat stereotyped. A detailed description of the regularities apparent in these behavior sequences is a necessary first step in the physiological analysis of female hamster agonistic behaviors.

EXPERIMENT 1: AGGRESSIVE BEHAVIOR ELEMENTS AND CHANGES IN RESPONSE-SEQUENCING WITH EXPERIENCE

Method

Subjects and Maintenance

Twelve random-bred female hamsters of the LVG:LAK strain were purchased from Lakeview Hamster Colony, Newfield, N.J. at 28-35 days of age (shortly after weaning). Hamsters were caged individually in metal cages measuring 25 x 18 x 18 cm or 30 x 30 x 18 cm. Purina rat chow and tap water were supplied ad libitum. Cabbage, sunflower seeds and Purina guinea pig chow were supplied at weekly intervals.

Lighting in the colony rooms varied according to a reversed 12-hour bright:12-hour dim cycle. Temperature fluctuations were attentuated, normal room temperature ranging between 20 and 25 degrees centigrade. During a period exceeding two years, more extreme temperature excursions (between 12 and 32 degrees centigrade) occurred as a consequence of occasional failures of thermostatic controls. Most (83%) of the experimental encounters were staged during a restricted period of two months (June-July), when experimental subjects were 110-160 days of age.

The Composition of Experimental Pairs

Variations in the external vaginal discharge (Orsini, 1961) allowed the estrous cycles of these intact females to be monitored. All twelve females which completed testing were followed through at least four complete estrous cycles before testing was begun, and maintained the same cyclic pattern throughout the duration of testing. Each female was assigned to one of six experimental pairs. The two members of each pair were cycling synchronously and were matched for body weight to within 10 gm of each other. The composition of each pair was held constant throughout testing.

Test Schedules

Each of six pairs of intact adult female hamsters experienced a series of eight 3-minute encounters in a clean neutral arena. The time of day of testing was held constant ($\pm$ 30 min.) for individual pairs and ranged between 3.5 and 6.5 hours after the start of the dim-light segment of the diurnal cycle. Tests were scheduled so that each pair experienced only one encounter per test-day. Successive tests typically were conducted at 3-day intervals, until each pair had experienced two encounters on each of the four days of the normal estrous cycle. Different pairs began the sequence of eight regularly spaced tests at different stages of the estrous cycle.

Only observations of the six nonestrous tests (two tests for each of the 3 nonestrous days of the 4-day cycle) experienced by each pair will be summarized in this chapter. The next chapter emphasizes contrasts in the aggressive behavior exhibited by these female-female pairs on tests during estrous, as opposed to nonestrous, days of the cycle.

Test Procedures

Encounters between female hamsters were staged in aluminum (floor and 3 walls) and glass arenas measuring 55 x 56 x 27 cm. An opaque plexiglas partition divided the arena into equal halves. Contestants were allowed a total of 30 minutes of pre-exposure to the arena prior to each encounter. This time was divided equally between the halves of the arena. In addition, a similar acclimatization period was provided 3 days prior to the first test in a series. This was included to familiarize subjects with the arena itself, and with the two 100-watt incandescent lights (mounted above and on opposite sides of the arena, each approximately 60-70 cm from the center of the arena floor) required for the filming of encounters (see below). On test days, these lights were activated only for the 3 minute duration of each encounter. Animals seldom reacted in any obvious way to the changes in light intensity accompanying light onset and offset. While we cannot totally exclude the possibility that these brief exposures to bright lights affected the social behavior of subjects, the similarity between behaviors observed here and those seen during informal observations in dim light renders this an unlikely source of major errors.

Females were not handled during the staging of encounters. Individuals were allowed to mount a small platform which was subsequently placed in the appropriate half of the arena. The female then was allowed to dismount and the platform removed. The same method of transporting subjects was employed at the midpoint of the 30 minute acclimatization period, when each female was transferred to the opposite half of the arena.

At the end of the pre-exposure period, the bright lights were activated, the partition removed, and the pair of females allowed to interact freely for approximately 3 minutes. The exact timing for each 3-minute encounter was begun the instant that the nose of either individual approached any part of its opponent's body to a distance of 1 cm or less, referred to as Nose-to-Nose in recognition of its typical form. Nose-to-Nose seemed to comprise a central element of a form of mutual investigation throughout which the muzzles of the two antagonists

remained in close apposition, with their vibrissae touching. However, in our scoring of agonistic behavior sequences, Nose-to-Nose served exclusively as a marker for the beginning of an experimental encounter. It was not scored except during this initial segment of staged social interactions.

Experimental encounters normally continued for three minutes from the initiation of Nose-to-Nose contact. However, if Flight (see definition below) occurred, it defined the end of an encounter, and an opaque partition was immediately inserted between the two contestants. Following each encounter, the arena was washed with acetone and hot water and the arena floor covered with clean cedar shavings.

Super-8 movies provided permanent records of the behaviors occurring during all experimental encounters. A Nizo S56 super-8 movie camera was mounted roughly 130 cm directly above the arena floor and single frames were exposed at a rate of approximately 2 per second. Short film segments of a clock immediately preceded the record of each day's tests and allowed the exact film speed to be determined during subsequent analysis. In addition to a view from directly above, a mirror mounted at a 45 degree angle outside the single glass wall of the arena (and within the view of the camera) provided a side view of all encounters.

Behavioral Categories

On the basis of extensive observations of hamster social encounters, the following definitions were found useful in the description and quantification of female agonistic behaviors.

Nose-to-Nose: Described above, Nose-to-Nose refers only to the initial instance of close social contact experienced by a pair following the removal of the arena's central partition. Thus, by definition, it always appeared as the first behavioral element in the sequence composing an encounter, and it was not scored again at any other point during the encounter. While Nose-to-Nose included instances in which the nose of one individual closely approached (to a distance of 1 cm or less) parts of its opponent's body other than her nose, its name indicates its typical appearance.

Upright: An individual in an Upright posture has neither forepaw in contact with the substrate and is within one body-length of her opponent. For this category to be scored, the opponent of an Upright female must herself have been in an Upright posture, or must have maintained a normal quadrupedal stance. In either case, the opponent of an Upright individual must have been located anterior to the vertical plane passing through the eyes of the Upright female. Subcategories of Upright postures sometimes were distinguished on the basis of whether the upright female's muzzle was closest to her opponent's head (Upright-Head) or trunk (Upright-Trunk).

Circle: This refers to a pattern of circular locomotion by both partners in which their bodies are antiparallel, the nose of each near the anogenital region of the other. To be scored as Circling, this motion must have been sustained through an angle of at least 90 degrees.

Follow: Follow refers to a slow pursuit of one individual by the other, the nose of the following animal usually remaining in close proximity to, or in contact with, posterior regions of its opponent's body.

Attack: Attacks are variable in form and may be launched from either an upright or a sideways posture (in the latter, only the forepaw nearest the opponent is off the substrate, the upper body being rotated to the side accordingly). In fact, upright and sideways postures both seem to form integral parts of most Attacks (Figure 1a). An Attack launched from an upright posture often elicits some form of sideways posture as a response. Conversely, an individual attacking in a sideways posture often evokes an upright stance on the part of its opponent. Thus, Attacks may be defined conveniently in terms of the simultaneous exhibition of upright and sideways postures by the two contestants. We have also required that the body of the upright individual be oriented approximately perpendicular to the trunk of her sideways opponent. Finally, for inclusion in this category an individual adopting an upright stance during an Attack must have had at least one forepaw in contact with some part of her opponent's body.

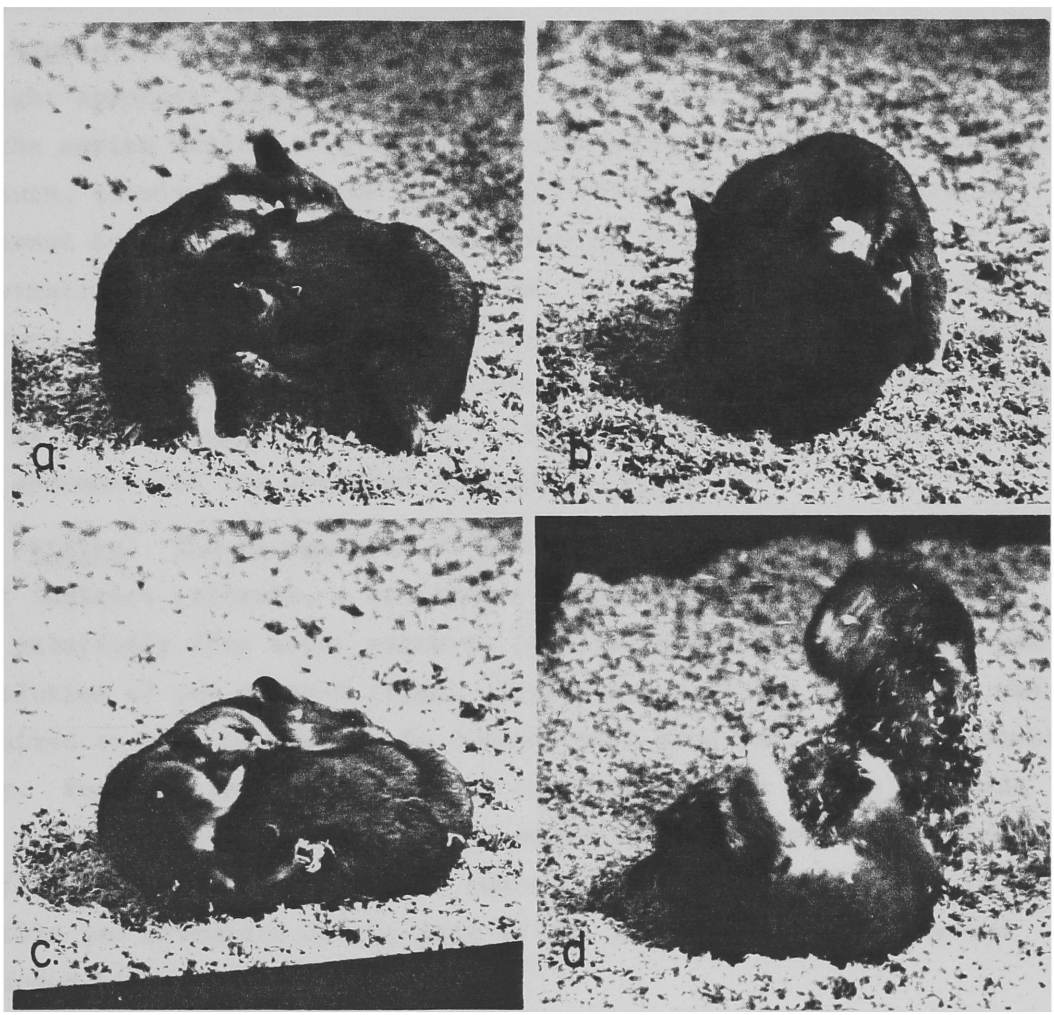


Figure 1. Typical examples of the following female hamster agonistic behaviors are illustrated in photographs of actual female-female encounters (range of exposure durations = 1/125 - 1/250 sec.): (a) Attack; (b) On Back; (c) Rolling Fight; and, (d) Fly-Away. These patterns are defined and described in greater detail in the text.

On Back: An individual engaged in On Back lies on its back or side (Figure 1b) with its opponent typically in an upright stance, oriented perpendicular to and hovering over the supine individual. A sequence of behaviors which commonly accompanied adoption of an On Back position of one hamster involved intermittent bites of the supine individual by its upright opponent (Aggressive Groom). During a bite, activity on the part of the supine hamster would cease, occasioning the release of the bite. In turn, termination of the bite would result in the resumption of movement by the supine individual, leading to another bite. Alternatively, an attacking female may be forced into an On Back posture, but may then continue to attack from this position. The less aggressive opponent of such an individual, though in an upright posture, may be fully occupied attempting to hold the attacking female away from its abdomen with its extended forepaws (a Pin).

Biting: The recognition of individual Bites often required somewhat indirect criteria, a concession necessitated by the variable and relatively fine motor patterns involved and the limited view and resolution of our super-8 films. Typically, a Biting individual was required to have her muzzle in contact with some part of her opponent's body. Further, some deflection of the skin or body of the opponent had to be detected at the point of muzzle-body contact. Additional confirmation of Biting activity sometimes was available in the form of visible jaw movements on the part of the individual delivering the presumed bites.

Rolling Fight: This pattern of violent aggressive behavior (Figure 1c) is highly distinctive and has been described as "fighting" or "locked fighting" by Kislak and Beach (1955), Dieterlen (1959), Bunnell et al. (1970), Payne and Swanson (1970) and Vandenbergh (1971). Typically, the bodies of the two antagonists are perpendicular to and wrapped tightly around each other, their abdomens in close apposition. Severe and persistent Biting, punctuated by frequent vocalization, may occur as the pair rolls wildly about. For the purposes of movie analysis, Rolling Fights have been considered to begin when both members of a

pair simultaneously rolled, or were thrown, onto their backs or sides. Rolling Fights then continued until either individual was able to maintain a bipedal or quadrupedal stance for at least four consecutive frames (approximately two seconds).

Fly-Away: Dieterlen (1959) has described an aggressive maneuver in which a hamster engaged in a Rolling Fight disengages himself rapidly with an explosive extension of the hindlimbs (Figure 1d). Such a Fly-Away provides an individual with a means of exiting rapidly from an unfavorable encounter. Moreover, the maintenance of a bite during this behavior can result in the severe wounding of an opponent. In either event, Fly-Away represents a behavior typical only of extremely aggressive encounters.

Flight: This refers to very rapid locomotion, at a rate of at least four body-lengths per second, directed away from the opponent. As described previously, Flight defined the end of any encounter in which it appeared. A fleeting individual was immediately separated from its opponent by an opaque partition. (If Flight did not occur, encounters continued for three minutes from the initiation of Nose-to-Nose contact.)

Lordosis: The extent of occurrence of Lordosis provided some indication of the sexual receptivity of test females. Rigid and prolonged immobility, a slightly depressed abdomen, elevation of the tail and a slight elevation of the head were characteristic of this distinctive posture.

Analysis

Behaviors were scored in the course of frame-by-frame analyses of movie films viewed on a small screen, using a modified Honeywell projector (Lafayette Super 8 Analyzer). The measures employed in most analyses of the agonistic behavior of experimental females included the latency and total duration of each of the following behaviors: Upright, On Back, Rolling Fight, Fly-Away and Lordosis. The latency of Flight and an estimate of the frequency of Biting (the percentage of 20-frame (11-second) blocks in which at least one bite occurred) also were tabulated. In analyses of the sequential ordering of behaviors,

instances of occurrence of the following (together with those above) were noted without regard for their duration or the durations of intervals separating them from adjacent behaviors: Nose-to-Nose, Circle, Follow, Upright-Head, Upright-Trunk and Attack.

The latency of any behavior corresponded to the interval between the initiation of Nose-to-Nose contact and the first occurrence of the behavior in question. Behaviors which failed to appear at all in the course of an encounter were assigned a latency equal to the total duration of the encounter. Thus, for most encounters, the maximum latency achieved by any behavior was 180 seconds. However, if Flight occurred during the encounter, interaction was terminated immediately and the contestants separated. In such cases, the time at which Flight intervened determined the latency assigned to behaviors which had not previously appeared during the encounter.

The total durations of behaviors were calculated on the basis of the number of movie frames on which the behavior was exhibited. If an encounter was terminated prematurely by Flight, observed durations of behaviors were normalized to 3 minutes, the usual encounter duration.

All behavioral categories described above specify the behaviors of both contestants at a particular instant. Because of this, and our emphasis on intragroup pairings in subsequent physiological analysis (Chapter III), it generally has not been necessary to distinguish between the two members of a fighting pair. Nevertheless, the durations of Upright and On Back contributed by each contestant (identified by clipping a small patch of fur prior to beginning the encounter series) were recorded, together with the identity of individuals engaging in Flight.

Reliability Checks

(a) Latency, Duration and Frequency Measures: The clarity of our definitions of some female hamster behaviors was checked by rescoring movies of 10 encounters. The encounters to be rescored included four involving intact nonestrous female pairs, maintained as described in a previous section. The remaining six encounters included two involving adrenalectomized-ovariectomized pairs and four involving hypophysectomized

female pairs. All of these females were maintained in a manner very similar to that previously described for intact pairs. All operates received desoxycorticosterone (Percorten pivalate, CIBA) replacement therapy (0.75 mg doses at 10-14 day intervals) throughout the period of experimentation. Both members of each pair also received one of the following hormone treatments: for adrenalectomized-ovariectomized pairs -- testosterone propionate (200 µg/day), estradiol benzoate (10 µg/day), progesterone (500 µg/day), or oil vehicle alone; for hypophysectomized pairs -- adrenocorticotrophic hormone (200 µg or 25 I.U./day), follicle-stimulating hormone (300 µg/day), luteinizing hormone (300 µg/day), prolactin (1 mg or approximately 20 I.U./day), or saline control.

Our main concern here involves a direct comparison of results obtained in the course of two scorings of a single encounter. Thus, variations among experimental pairs in physiological manipulations and hormone treatment may be ignored, since each pair acts as its own control. While a detailed discussion of the conditions under which operated pairs were maintained will be reserved for the next chapter, their immediate consideration provides a direct demonstration that procedures summarized here yield reliable results in a variety of experimental situations.

The ten encounters selected for reliability checks of latency, duration and frequency measures of female hamster aggression were staged over a 13 month period, and the most recent one was rescored at least 6 months after its initial viewing. Procedural details common to all of these encounters have been described in a previous section of this chapter.

(b) Behavioral Sequences: In the analysis of the ordering of hamster agonistic behaviors during encounters, behavioral sequences were broken down into 2-act sequences (e.g., see Table II). Since the operations, and some of the behavioral categories, appropriate to this form of analysis differ from those in analyses focusing on the latencies, durations or frequencies of a few selected behaviors (previous section), some additional checks on the reliability of experimental procedures seemed necessary.

Seven encounters were selected randomly from the total of 36 involving pairs of intact nonestrous females matched for body weight and estrous cycle. The sequences of behavior observed during frame-by-frame analysis of movies of these encounters were retabulated. Recheck scoring was conducted 4-21 days after the initial viewing of the same encounter. Direct comparison of original-recheck pairs falling at the extreme ends of this range indicated that, at least within this relatively narrow range of intervals, differences between original and recheck pairs of analyses did not appear to depend upon the length of the intervening interval.

Statistics

Tests of the reliability of our procedures for scoring hamster aggressive behaviors have been evaluated on the basis of the Pearson product-moment correlation coefficient, together with tests of the significance of differences between observed correlation coefficients and chance values of zero (Edwards, 1966). The nonparametric sign-test (Siegel, 1956) has been employed in evaluating differences in roles adopted by individual pair members during encounter series. The t-test for dependent means (Edwards, 1966) has been used in comparisons of transitional frequencies among behaviors at different stages in a series of encounters. All statements regarding the statistical significance or chance probabilities of observed differences have been based on two-tailed tests of significance.

Results

Reliability Checks

(a) Latency, Duration and Frequency Measures: Data bearing on the reliability of some of our aggression measures are summarized in Table I. Product-moment correlation coefficients (Edwards, 1966) indicate a highly significant degree of association between the original and repeat scores of nearly all measures of female aggressiveness. Latencies of Biting provide the only major exception. Original and repeat latencies of this behavior are not associated to a significant extent ($r = .17$, $p > .10$). This lack of a substantial correlation suffices to eliminate Biting latency from serious consideration as a reliable

Table I: Checks on Reliability of Measures of Female Hamster Aggression

	Median Latency (sec.)			Mean Durations (sec.) and Frequency (%)**		
Behavior	Original Score	Repeat Score	*** r	Original Score	Repeat Score	*** r
Upright	2.9	2.4	1.00*	46.5	40.6	.97*
On Back	50.7	27.3	.98*	31.7	33.4	1.00*
Biting	20.1	15.7	.17	23.2	26.2	.78*
Rolling Fight	179.8	180.2	1.00*	2.77	1.78	.93*
Fly-Away	179.8	180.2	.98*	.44	.65	.99*
Flight	179.8	180.2	1.00*			

* $p < .01$

** For all behaviors except Biting, the measure of the intensity of aggression was the duration (sec.). The frequency of Biting was estimated in terms of the percentage of 11-second blocks in which at least one Bite occurred.

*** r = Pearson's product-moment correlation coefficient (Edwards, 1966).

indicator of levels of hamster aggression under our testing regime. On the other hand, original and repeat frequencies of Biting are more closely related ($r = .78$, $p < .01$), and approach the very high degree of association exhibited by all measures based on behaviors other than Biting ($r \geq .93$, $p < .01$).

High correlations between the original and repeat scores achieved by a variety of aggression measures suggest that the procedures we have utilized in the scoring of movies of hamster encounters yield highly reliable results. Direct comparisons of median latencies, and mean durations, observed during the first and second viewings of encounters confirm this conclusion (Table I). Further, average absolute differences between original and repeat duration scores are small, ranging between 0.2 and 6.2 seconds. Similarly, average absolute differences between

scores on the first and second determinations of median latencies also are small, accounting for less than 0.6 percent of the latency originally attributed to that behavior. Finally, the fact that infrequent behaviors (e.g., Flight) often were assigned 180-second latencies has not significanly affected any measures of reliability summarized above. For these behaviors, high degrees of agreement between original-repeat pairs of latencies, or durations, also were apparent in analyses restricted to the few encounters during which the behavior in question actually occurred.

(b) Behavioral Sequences: Tables summarizing the average transitional frequencies (the frequencies of each of 81 two-act sequences such as included in Table II) obtained during initial and subsequent viewings of seven encounters were compiled and compared. Product-moment correlation coefficients indicated an extremely high degree of association between the original and repeat determinations of all 81 transitional frequencies ($r = .97$, $p < .01$). Even discounting the 41 two-act sequences which failed to occur within this limited sample of encounters (and thus contributed transitional frequencies of zero during both scorings) original and recheck transitional frequencies still were strikingly similar ($r = .95$, $p < .01$). Finally, the average difference between original and recheck determinations of transitional frequencies, expressed as a percentage of the original transitional frequency, was very small (3.3%). Thus, as in the cases of latency, duration and frequency measures, procedures which we have developed for the description of behavior sequences occurring during encounters between nonestrous female hamsters yield highly reliable results.

Informal Description of Typical Encounter

Figure 2 summarizes our informal description of the sequence of social behaviors characteristic of encounters between nonestrous adult female hamsters. This scheme is based upon observations of many such encounters and on the informal analysis of their records on movie film.

The initial stage of an aggressive behavior sequence usually involves mutual investigation, with the noses of the antagonists in apposition and their vibrissae touching (Nose-to-Nose in Figure 2).

IMPRESSIONISTIC SCHEME OF FEMALE-FEMALE HAMSTER ENCOUNTERS

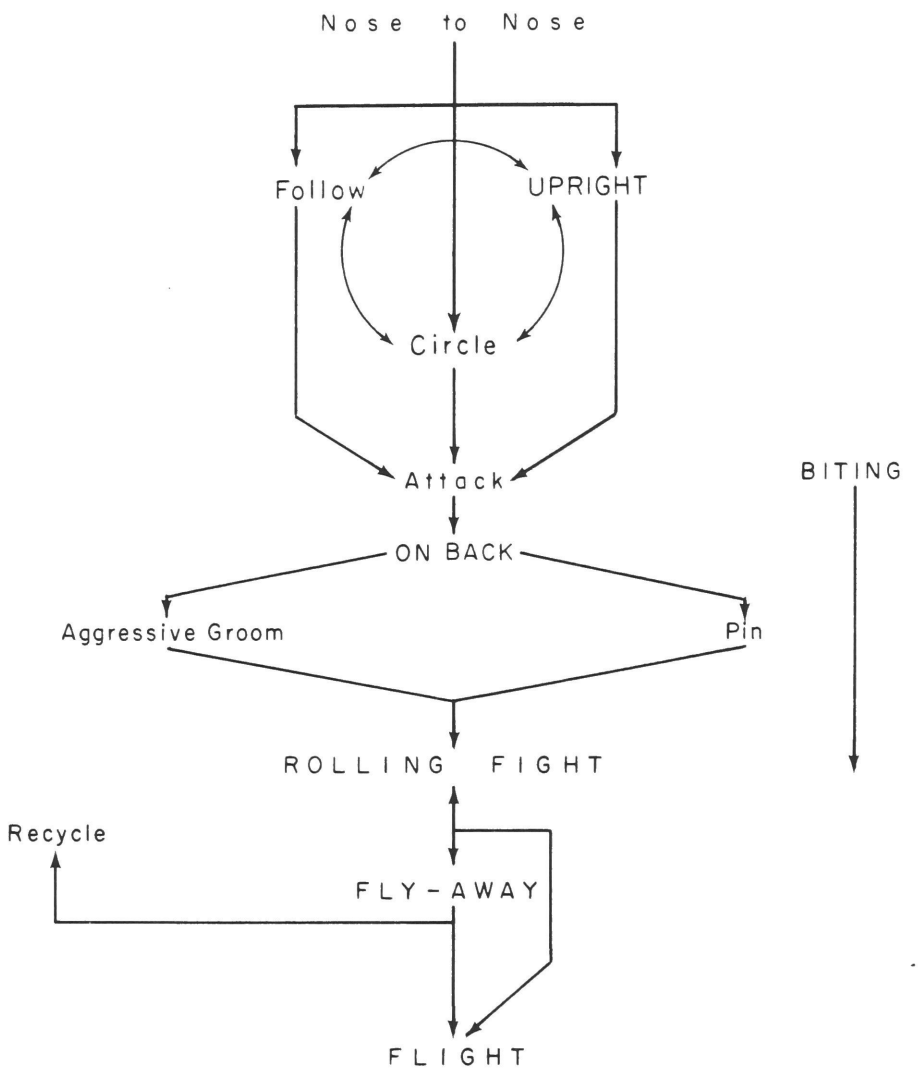


Figure 2. Sequence of agonistic behavior patterns typically observed during a brief encounter between anestrous female hamsters. Capitalized terms denote behavior patterns selected as quantitative measures of aggressiveness. Thin lines connect patterns thought to represent similar levels of aggressiveness. Individual responses included in this scheme are defined in the text.

Following this initial contact, one or both females may continue social investigation and mild agonistic behaviors in an Upright posture or while Circling or Following. These three activities all seem to occupy similar positions in the hierarchy of female agonistic behaviors: any of the three patterns may immediately succeed initial Nose-to-Nose investigation; they may alternate extensively among themselves; and, any may serve as the immediate prelude to an Attack.

Among behaviors included in Figure 2, Attacks clearly represent a relatively complex form of interaction. Most other behaviors could be defined in an objective, but simple, manner which allowed them to be scored easily from the 2 frame-per-second movies used routinely to quantify the aggressiveness of hormonally manipulated females (Chapter III). In contrast, the recognition of individual Attacks was a tedious process. Thus, it is significant that alternative routes of attack have, as common end points, the creation of a situation in which one individual is lying on its back or side (On Back in Figure 2). Instances of On Back were much easier to quantify than individual Attacks. This, and the intimate relationship of the two, strongly recommends the On Back position as a convenient indicator of moderate to high levels of aggression.

Biting is variable with respect to force and with respect to order of occurrence in the chain of hamster agonistic behaviors (Figure 2). Substantial frequencies of Biting usually occur first in association with Attacks, but are even more closely associated with subsequent behaviors indicative of higher levels of aggressiveness.

Gradually increasing intensities of Biting, such as might occur in the course of Aggressive Grooming or during an unsuccessful Pin, may provoke a Rolling Fight (Figure 2). Rolling Fights sometimes were terminated abruptly by Fly-Aways. However, during particularly violent encounters, Fly-Away often was followed by an immediate resumption of rolling fighting, or by a "recycling" to some earlier phase of the agonistic sequence (Figure 2). An alternative successor to Fly-Away, or a Rolling Fight, was Flight, rapid and persistent retreat by one individual, with its opponent often in close pursuit. If Flight occurred, it defined the end of an encounter and the contestants were immediately separated and returned to their home cages.

For simplicity, references in this informal description, and in Figure 2, to behavioral transitions involving "recycling" to behaviors characteristic of an earlier stage of the encounter are incomplete. In fact, nearly all transitions between adjacent behaviors in Figure 2 were bidirectional. More complex transitions among nonadjacent behaviors included in Figure 2 also were frequent and varied.

Figure 2 clearly represents an idealized description of the behaviors and behavioral sequences characteristic of encounters between nonestrous female hamsters. Its primary object was to suggest distinctive behaviors and postures which correspond to a variety of different levels of female hamster aggressiveness and which, therefore, might provide valid quantitative indices of aggression in experimental situations.

The Validity of Selected Measures of Female Hamster Aggressiveness

Capitalized terms in Figure 2 denote behaviors which we have selected as quantitative measures of female hamster aggressiveness. Their appropriateness depends upon how well the impressionistic scheme (Figure 2) as a whole depicts the sequence of events which actually occur during brief female-female encounters. For example, Figure 2 predicts a particular order in which behaviors may be expected to appear during a typical encounter. Thus, the relative latencies of behaviors selected as measures of aggressiveness provide some indication of the accuracy with which the scheme describes the agonistic behavior of female hamsters.

Each of six pairs of intact female hamsters was tested, as described previously, in six encounters on nonestrous days of the normal 4-day cycle. These were different encounters than those which generated the scheme. Behavior patterns described above have been listed in the central portion of Figure 3 in the order in which they appear in the scheme of Figure 2. The relative latencies of these behaviors, indicated by the relative lengths of horizontal histograms in Figure 3, provide convincing support for the scheme. Behaviors appearing early in the scheme, and considered to be indicative of relatively low levels of aggressiveness, have short latencies. Conversely, behaviors considered to represent intense aggression, and occupying later positions within the scheme, are

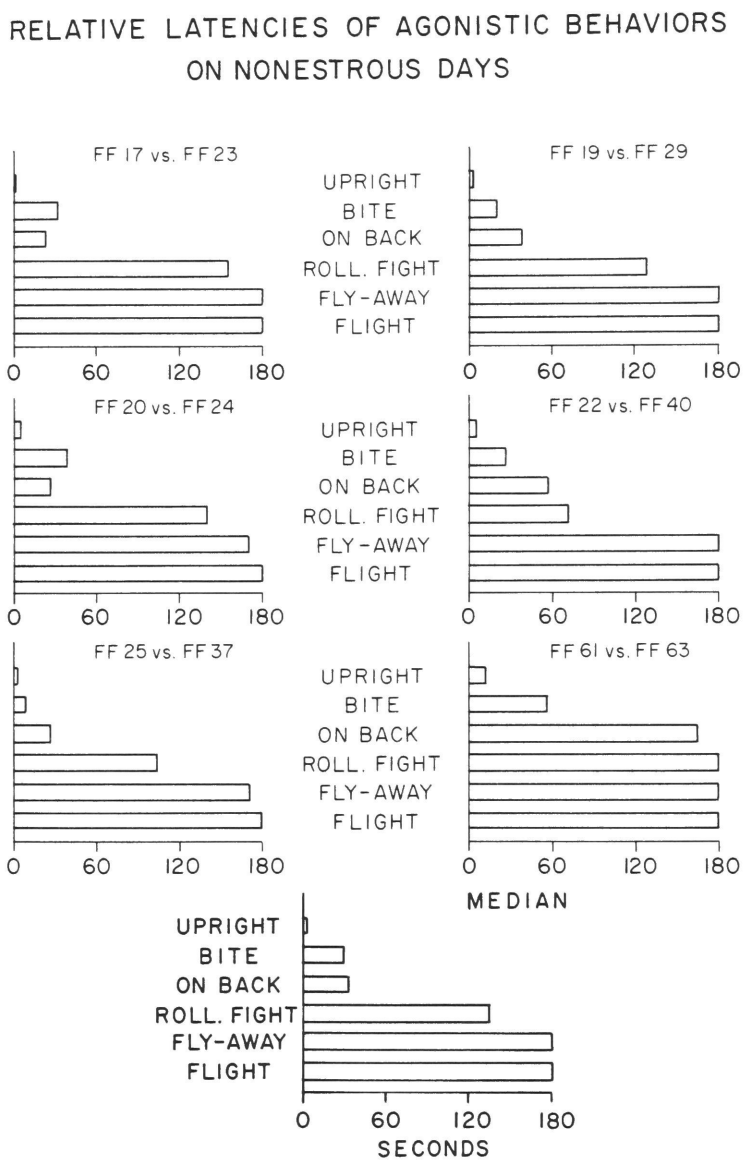


Figure 3. Observed latencies of agonistic behaviors are compared with the order of occurrence predicted by the scheme of Figure 2. Vertical lists of our behavioral measures in the central part of this figure show the expected order of occurrence based on the scheme. Data included summarize all nonestrous encounters experienced by each of 6 pairs of intact adult female hamsters (e.g., FF17 vs. FF23), and the median latencies across all 6 pairs (bottom). Encounters lasted 3 minutes or less (maximum latency, 180 sec.).

characterized by relatively long latencies. Thus, to a first approximation, the description inherent in Figure 2 seems to reflect accurately the chain of behavioral elements characteristic of encounters between adult nonestrous hamsters.

The scheme of Figure 2 also attempts to specify those behaviors which, at all points during an encounter, are most likely to precede and to follow any particular behavior. For example, adoption of the On Back position should be preceded by an Attack. Transitions to On Back from any other behavior should be relatively infrequent. Similarly, the scheme predicts that the development of an intense Rolling Fight requires the prior performance of the On Back posture. Alternative paths leading to this advanced stage of an aggressive interaction should be much less prominent.

The relative frequencies of transitions among 9 behaviors included in our impressionistic scheme are summarized in Figure 4. Here, line thickness is directly related to mean transitional frequency: Very thick lines correspond to very frequent transitions between behaviors (averaging 2 transitions per encounter or more), while very thin lines denote relatively infrequent transitions (on the order of 0.2-0.3 per encounter). Transitions among different behaviors have been emphasized in that all instances in which a behavior pattern was followed immediately by a reoccurrence of that same pattern have been excluded. A numerical summary of average transitional frequencies observed during these encounters is included in Table II. Finally, transitions among agonistic behaviors also have been described in terms of the percentage of instances of a given behavior pattern which were preceded (Table III) and followed (Table IV) by each of the behaviors included in our inventory.

Concentrating first on the relatively simple summary of mean transitional frequencies depicted in Figure 4, some general points of similarity between this description and the impressionistic scheme (Figure 2) are readily apparent. For example, the spatial organization inherent in the impressionistic scheme suggested that female hamster encounters might be subdivided into more or less distinct phases. An initial phase seemed to be characterized by variable transitions

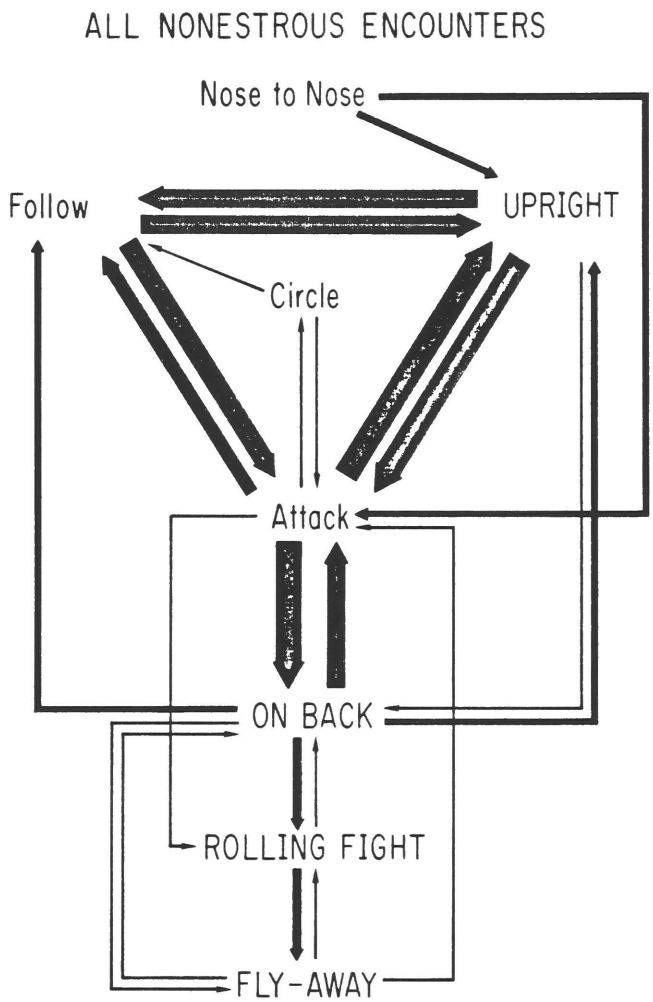


Figure 4. Graphic summary of the mean transitional frequencies (mean number of transitions per encounter) among the hamster agonistic behaviors included in the scheme of Figure 2. Each of 6 pairs of intact female hamsters experienced a series of six 3-minute encounters (two on each of the three nonestrous days of the cycle). This figure summarizes the mean transitional frequencies over the entire series of six encounters. Average transitional frequencies also have been described for the First (Figure 5), Fourth (Figure 6) and Sixth (Figure 7) nonestrous encounters in the series of six. In each figure, line thickness is related to mean transitional frequency according to the following code:

.15 - .34/encounter

.35 - .54

.55 - .74

.75 - .94

.95 - 1.99

$\geq$ 2.00

Table II: Behavioral Transition Frequency

(mean number per pair per encounter), All Nonestrous Encounters

FROM: \ TO:	Circle	Follow	Upright-Head	Upright-Trunk	Attack	On Back	Rolling Fight	Fly-Away	Flight	Total
Nose to Nose	.08	.08	.36	.06	.36	.03	0	0	0	.97
Circle	.03	.22	.03	.03	.25	.06	0	0	0	.62
Follow	.14	.44	.81	.44	1.25	.11	.03	0	0	3.22
Upright-Head	0	.67	.39	.39	1.36	.11	0	0	0	2.92
Upright-Trunk	.06	.44	.56	.64	1.28	.17	.03	0	0	3.18
Attack	.19	.94	.81	1.28	3.17	2.48	.22	.06	0	9.15
On Back	.14	.42	.12	.36	1.28	1.33	.58	.17	.03	4.43
Rolling Fight	0	0	.03	.03	.08	.22	0	.61	.03	1.00
Fly-Away	0	.06	.03	.03	.31	.23	.17	0	0	.83
Total	.64	3.27	3.14	3.26	9.34	4.74	1.03	.84	.06	26.32

Table III: The Percentage of Occurrences of a Behavior which were Preceded by Each of Nine Behaviors, All Nonestrous Encounters

'RECEDED BY:	Nose to Nose	Circle	Follow	Upright-Head	Upright-Trunk	Attack	On Back	Rolling Fight	Fly-Away	N*
	13	4	22	0	9	30	21	0	0	23
	3	7	14	20	14	29	12	0	2	118
-Head	12	1	26	13	18	26	4	1	1	112
Upright-Trunk	2	1	14	12	20	39	11	1	1	117
Attack	4	3	13	15	14	34	14	1	3	336
On Back	1	1	2	2	4	52	28	5	5	170
Rolling Fight	0	0	3	0	3	22	56	0	16	37
Fly-Away	0	0	0	0	0	7	20	73	0	30
Flight	0	0	0	0	0	0	50	50	0	2

* N = The total number of occurrences of each behavior listed on the left.

Table IV: The Percentage of Occurrences of a Behavior which were Followed by Each of Nine Behaviors, All Nonestrous Encounters

R: \ FOLLOWED BY:	Circle	Follow	Upright-Head	Upright-Trunk	Attack	On Back	Rolling Fight	Fly-Away	Flight	N*
Nose	9	9	37	6	37	3	0	0	0	35
	5	36	5	5	41	9	0	0	0	22
	4	14	25	14	39	3	1	0	0	116
Upright-Head	0	23	13	13	47	4	0	0	0	105
Upright-Trunk	2	14	18	20	40	5	1	0	0	114
Attack	2	10	9	14	35	27	2	1	0	329
On Back	3	9	3	8	29	30	13	4	1	159
Rolling Fight	0	0	3	3	8	22	0	61	3	36
Fly-Away	0	7	3	3	38	28	21	0	0	29

* N = The total number of occurrences of each behavior listed on the left.

restricted to a group of relatively mild agonistic behaviors (Follow, Upright and Circle in Figure 2). A subsequent phase differed in its emphasis on more stereotyped transitions among more severe aggressive behaviors (e.g., On Back, Rolling Fight and Fly-Away). The results of more quantitative analysis (Figure 4) support this interpretation. However, in contrast, to earlier impressions (Figure 2), behavioral transitions observed in most encounters do not achieve a very high degree of stereotypy before entering the sequence of On Back to Rolling Fight to Fly-Away. That is, our original description of female hamster fights (e.g., Figure 2) emphasized the intimacy of inter-relationships among Circling, Following and Upright postures. In fact, Circling appears to be a very infrequent behavior pattern and the most conspicuous cluster of highly interconnected behaviors includes not only Following and Upright postures, but also Attacks (Figure 4). Figures 2 and 4 also are in substantial agreement regarding many very specific characteristics of the behavior sequences occurring during female-female encounters. For example, the adoption of an On Back posture is preceded most frequently by an Attack (Table III, also Tables IV and V). Similarly, the development of a violent Rolling Fight does seem, in most cases, to require On Back as an immediate antecedent (Tables III-V). More generally, the scheme of Figure 2 specifies only a fraction of the two-act sequences possible among behaviors in our inventory (see Table II). Thus, if the mean frequency of occurrence of transitions (two-act sequences) which are included in the impressionistic scheme were significantly greater than the average frequency for transitions not specified in Figure 2, the scheme would seem to have identified the most likely transitions among hamster agonistic behaviors with some consistency. In fact, the mean frequency of transitions which are included in the impressionistic scheme was 0.63 occurrences per encounter. In contrast, transitions not specified in Figure 2 occurred at a mean rate of only 0.32 per encounter ($p < .01$, t-test, two-tailed).

In some computations summarized above, the impressionistic scheme of Figure 2 has been evaluated as if it had been intended as a complete description of behavior sequences occurring during female-female

encounters. In fact, major sorts of transitions (e.g., the immediate recurrence of the same behavior, and various modes of recycling to behaviors characteristic of earlier phases of the encounter) were excluded from consideration in Figure 2. Since these transitions account for most of those omitted from Figure 2, and since other errors of inclusion or emphasis are virtually absent, the impressionistic scheme would seem to have achieved its primary objectives and to constitute an accurate, though simplified, description of the behavior sequences characteristic of female hamster agonistic interactions.

Changes in Aggressive Behavior over Successive Encounters

We have suggested that some discrepancies among the results of various investigations of female hamster aggression (e.g., Payne & Swanson, 1971c and 1972c; Vandenbergh, 1971) may be partially attributable to differences in the experimental histories of animals participating in these studies. This suggestion, however, presupposes the existence of dramatic short-term changes in female hamster fights with increasing social experience. Agonistic response sequences exhibited by juvenile Stichaeus punctatus (Farwell & Green, 1973) change significantly over a three hour period. The early appearance of violent aggressive behaviors and the consequent rapid formation of rigid dominance orders characteristic of uninterrupted hamster encounters suggest that hamster response sequences might vary over minutes instead of hours (also see Allin & Banks, 1968; Lerwill & Makings, 1971; and, Reynierse, 1971). A simple comparison of response sequences observed at different points within our series of six nonestrous encounters might provide some insight into this issue.

During the first 3-minute encounter experienced by a pair of nonestrous females, the mean frequency of Attacks (18.8/encounter) was significantly greater than the average Attack frequency for the entire series of six encounters (overall Attack frequency = 9.3/encounter; $p = .004$, t-test). Transitions to and from Attacks also were relatively frequent during the first encounter (compare Figures 4 and 5). Accordingly, the frequency with which the On Back position was exhibited during initial pairings exceeded that observed over the series of six

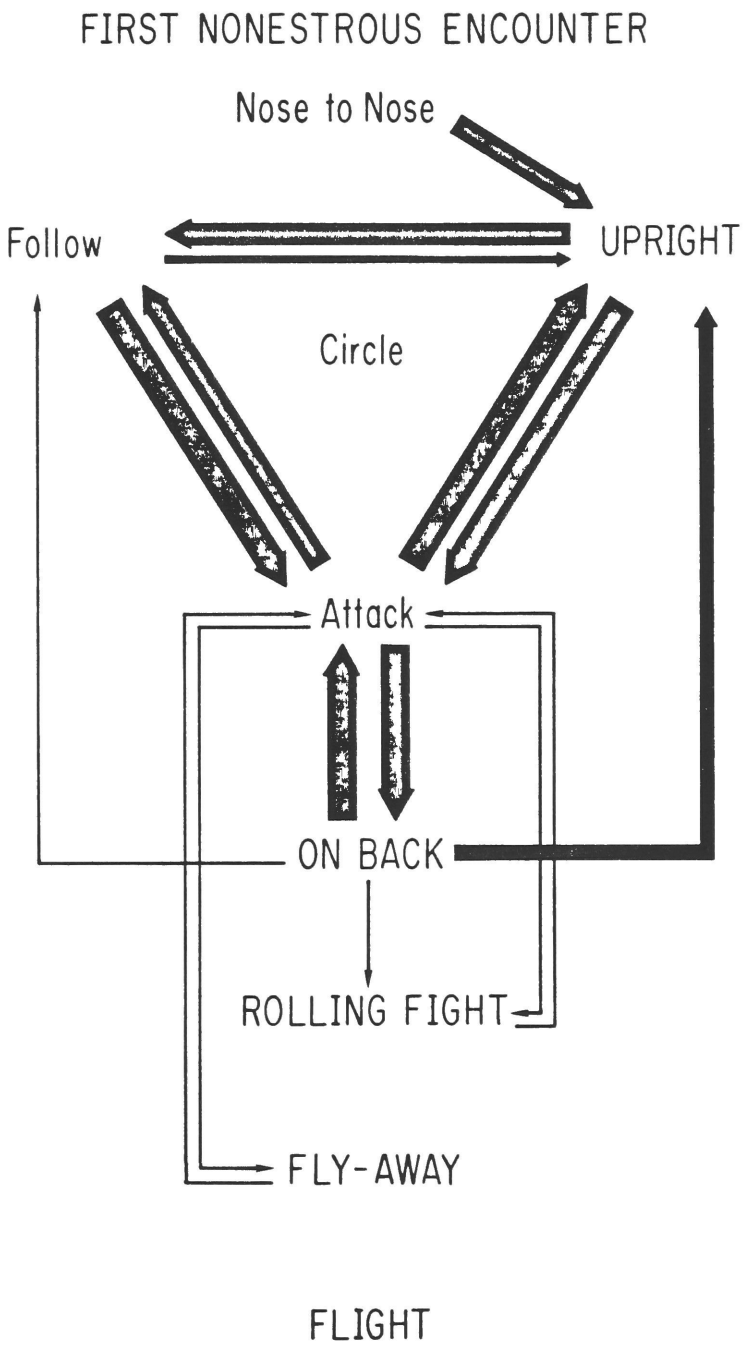


Figure 5. Graphic summary of the mean transitional frequencies among hamster agonistic behaviors observed in the first in a series of six female-female encounters. See Figure 4 for details and for the key relating line thickness to transitional frequency.

encounters (5.8/encounter during initial pairings versus 4.7/encounter overall, $p = .08$, t-test). In contrast, transitions from On Back to Rolling Fights were relatively infrequent during initial pairings, while those by which On Back "recycles" to more mild behaviors were relatively frequent (Figures 4 and 5; see also Tables II and IV). An important consequence of this difference is that, of the many behavioral transitions exhibited by naive females, virtually all avoid the most violent behavioral elements. A high degree of stereotypy is prominent as participants in initial encounters apparently engage extensively in a regular chain of social investigatory and mild agonistic behaviors.

At approximately the midpoint of the encounter series (the fourth nonestrous encounter) the frequencies and sequencing of behaviors most closely resembled the average pattern for the entire series of six encounters (Figures 4 and 6). Compared with the first pairing, fewer transitions were observed during the fourth encounter (mean total number of two-act sequences during encounter 4 = 24.5, as opposed to 35.9 during encounter 1; $p = .08$, t-test). This decrease in overall transition frequency could have stemmed, in large part, from a pronounced decline in Attack frequency (from 18.8 to 8.5 Attacks/encounter during encounters 1 and 4, respectively; $p < .002$, t-test). Since the percentages of Attacks that proceeded to On Back were similar at both points in the encounter series, the frequency with which the On Back position was exhibited also declined somewhat between the first and fourth pairings. However, despite a lower frequency of occurrence of On Back, an elevated frequency of transitions from On Back to Rolling Fight resulted in an increased number of Rolling Fights in the fourth encounters (compare Figures 5 and 6). Thus, the most dramatic and significant difference between the behavior sequences typical of the first and fourth pairings seems to be a considerable increase in emphasis on more violent and potentially damaging behaviors in the later encounters. Probably as a consequence of asynchronous changes in aggressiveness by different pairs, this trend toward the accentuation of relatively violent behavioral elements is more consistent in comparisons of encounter 1 with a group of intermediate encounters (2-5) from the series of six. In fact, each of the following indices of relative aggressiveness increased

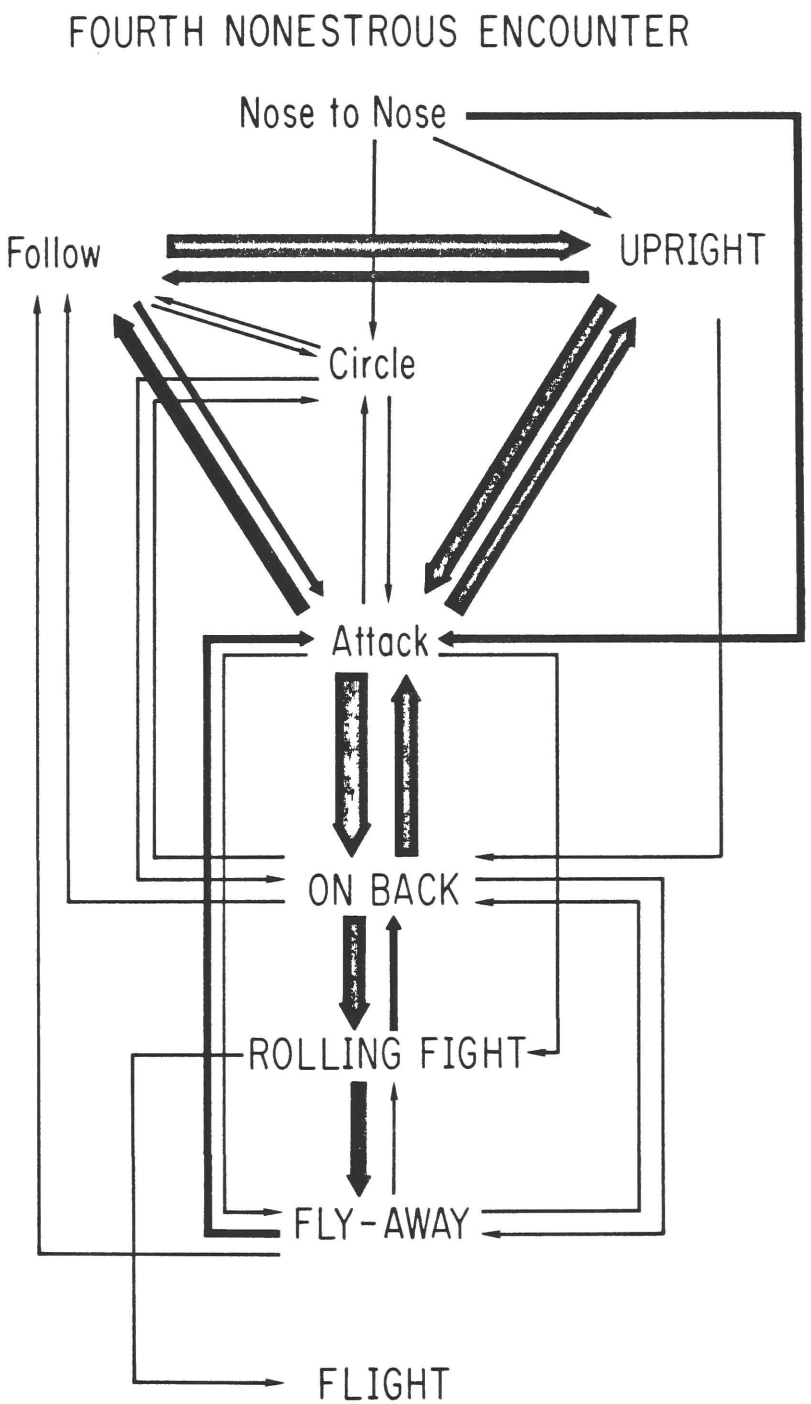


Figure 6. Graphic summary of the mean transitional frequencies among hamster agonistic behaviors observed in the fourth in a series of six female-female encounters. See Figure 4 for details and for the key relating line thickness to transitional frequency.

significantly between encounter 1 and encounters 2-5: the sum of Rolling Fights, Fly-Aways and Flights (from 0.50/encounter during encounter 1 to 2.37/encounter during encounters 2-5, $p = .048$, t-test); the ratio of Rolling Fights to Attacks (from 0.02 to 0.15 during encounters 1 and 2-5, respectively, $p = .044$, t-test); and, the ratio of Rolling Fights to On Backs (from 0.07 to 0.30 during encounters 1 and 2-5, respectively, $p = .054$, t-test). Finally, together with this increase in emphasis of relatively violent agonistic behaviors, the number and variety of behavioral transitions seems to increase prior to or during the intermediate meetings in an encounter series (e.g., compare Figures 5 and 6). Nevertheless, considerable stereotypy still is evident in response sequencing observed during the fourth nonestrous encounter, particularly among behaviors indicative of relatively high levels of aggression (Figures 5 and 6).

During the sixth nonestrous encounter experienced by female-female pairs, the average frequency at which behavioral transitions appeared was lower than at any preceding point in the encounter series (15.4 transitions/encounter during encounter 6, compared with 35.9 and 24.5/encounter during encounters 1 and 4, respectively; $p \leqslant .012$ in each case). This decline was evident in all behaviors indicative of high levels of aggression (Figure 7), but was especially marked in the case of Attacks (3.0 Attacks/encounter in encounter 6, versus 18.8 and 8.5 Attacks/encounter during encounters 1 and 4, respectively; $p < .002$ in each case). The degree of stereotypy in the sequencing of behaviors also had decreased by the time pairs of females met for their sixth 3-minute encounter. Pairs engaged in any particular behavior exercised greater variety in their choice of transitions, or paths, to immediately succeeding behaviors (note the many thin lines in Figure 7). The drastically decreased intensity and increased variability of social interactions occurring during these final pairings may signal fundamental changes in the character of social relations typical of experienced, as opposed to socially naive, pairs.

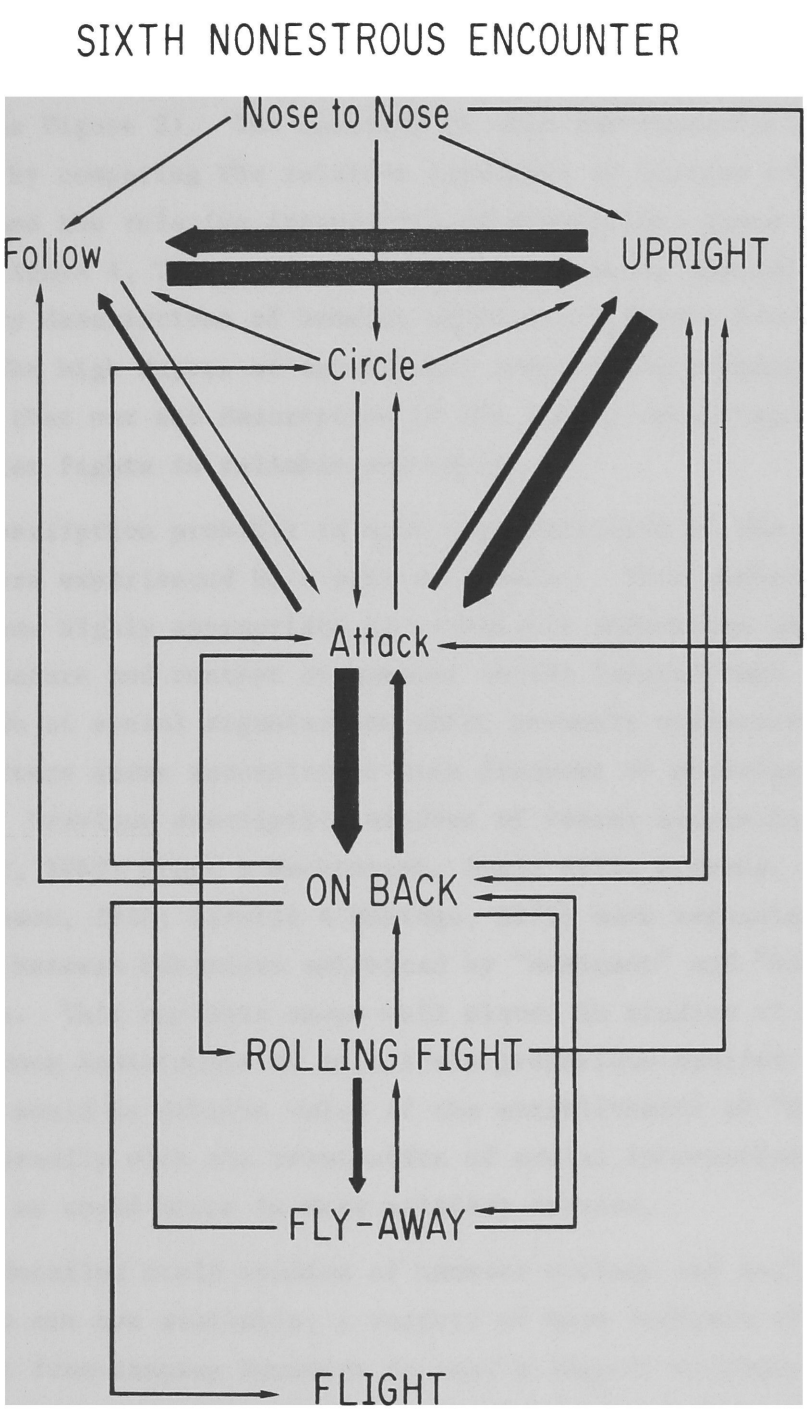


Figure 7. Graphic summary of the mean transitional frequencies among hamster agonistic behaviors observed in the sixth in a series of six female-female encounters. See Figure 4 for details and for the key relating line thickness to transitional frequency.

Discussion

We have generated a preliminary flowchart identifying behaviors and sequences of behavior typical of encounters between nonestrous female hamsters (see Figure 2). The accuracy of this impressionistic scheme has been tested by comparing the relative latencies of various behaviors (Figure 3) and the relative frequencies of transitions among these behaviors (Figure 4, Tables II-IV). In this process, several complementary descriptions of hamster agonistic behavior have been compared. The high degree of consistency among these accounts leads us to conclude that our net description of the events occurring during female hamster fights is reliable and valid.

This description probably is most representative of the initial few encounters experienced by a pair of females. This emphasis, however, seems highly appropriate to reasonable inferences regarding the natural structure and context of hamster social interactions. The solitary mode of social organization which probably characterizes free-ranging hamsters seems inconsistent with frequent or prolonged social encounters. Previous descriptive studies of rodent agonistic behaviors (e.g., Grant, 1963; Grant & Mackintosh, 1963; Allin & Banks, 1968; Payne & Swanson, 1970; Lerwill & Makings, 1971) have emphasized differences between behaviors exhibited by "dominant" and "submissive" participants. This emphasis seems well placed in studies of social relations among individuals of relatively gregarious species. Clearly, however, it would be dubious value if the establishment of "dominance" coincided normally with the termination of social interactions (e.g., by Flight), as could occur in more solitary species.

While detailed field studies of hamster ecology and social organization are not available, a variety of more indirect observations suggest that free-ranging hamsters do lead a largely solitary existence. The most convincing indirect evidence is simply the extreme intolerance of conspecifics exhibited by domestic hamsters of both sexes and most endocrine states. Violent fighting in pairs of any composition may be observed even when encounters are prolonged and repeated frequently over long periods of time (e.g., Payne & Swanson, 1970). At least in same-sex

pairings, any stabilization of relationships that occurs following repeated encounters seems to depend upon persistent avoidance of one participant by the other member of the pair (unpublished observations; also see Johnston, 1974c). Fighting is not highly ritualized and may result in serious injury or death. Even when death does not result directly from fighting, the polarity of relations among confined hamsters is such that dominant individuals may be able to exercise complete control over access to food within the enclosure (see Johnston, 1974c).

The high degree of social intolerance described above applies to domestic hamsters even in large 16 x 20 foot enclosures (Johnston, 1974c). This suggests that free-ranging individuals might occupy exclusive areas and live separated by considerable distances. Further, at least with respect to some laboratory measures of reproductive and aggressive behaviors, wild-caught hamsters are quite similar to representatives of laboratory strains (Murphy, 1971, also personal communication). While subtle alterations in acoustic or olfactory communications might be very difficult to detect during such observations, high levels of social intolerance such as those exhibited by domestic hamsters would seem quite unmistakable.

Other evidence suggesting that hamsters normally adopt a solitary mode of existence includes Aharoni's (1932) assertion that individuals live solitarily in self-constructed burrows. Further, a similar social organization has been described for the related European hamster (Cricetus cricetus) in semi-natural environments (Eibl-Eibesfeldt, 1953). Here, individuals defend territories surrounding their burrows, and males are permitted to enter the burrow of an estrous female only for the brief period during which mating occurs.

Until more detailed field observations of natural populations of hamsters become available, it seems most reasonable to presume a social organization in which individuals live solitarily, each scentmarking (Johnston, 1970a; Ralls, 1971) and possibly defending a remote area surrounding a home burrow. Encounters between spatially-separated conspecifics might then be rare, and at least among same-sex individuals,

transient. Some characteristics of such hypothetical encounters have been incorporated in our experimental tests. In particular, encounters, and encounter series, have been relatively brief, and encounters have been terminated immediately following attempted flight by either individual.

These manipulations reflected an effort to avoid the creation of significant differences between the members of individual female-female pairs. Nevertheless, pronounced changes in response frequency and sequencing have been demonstrated (Figures 5-7) even within the limited confines of our encounter series. In general, this suggests that rapid changes in the organization of hamster agonistic behaviors are difficult to avoid and constitute a factor worthy of some consideration in interpretations of effects of physiological manipulations upon hamster aggression.

We may inquire as to whether such changes in behavior sequences of necessity go hand-in-hand with the development of polar dominance relations. That is, to what extent have procedures adopted here been successful in avoiding a pronounced dichotomy between "dominant" and "submissive" pair members? Changes in the patterning of behavior elements could, after all, reflect simple habituation, rather than dominance.

Thirty encounters were reviewed and were classified according to the presence or absence of a clear difference in the extent to which one individual followed the other. The results of this categorization then were compared with a variety of measures including the presence or absence of Rolling Fights and Flight, and differences between pair members in On Back durations accumulated during the encounter. From this comparison, it is clear that the procedures we have employed in the staging of social encounters have not eliminated all differences between the members of each pair. In particular, we have found a persistent correlation between following and the performance of On Back. In encounters involving five female-female pairs, the "followee" in the encounter series tended to perform On Back with longer durations than the "follower" ($p = .06$, sign-test).

Stable differences in the extent to which one individual follows its partner are suggestive of dominance. The observation that such differences are correlated with differences in On Back duration suggests further that On Back is primarily (though not exclusively) a response exhibited by an individual that consistently withdraws from, and thus seems submissive to, her opponent. However, a correlation between On Back and following could stem from a variety of factors other than their mutual dependence on relative dominance. For example, at least some instances of On Back may represent "startle" responses to unexpected contact with ones hindquarters by a following individual. Thus, it seems essential to recognize that "dominance" is an elusive term, and that "relative dominance" may be critically dependent upon the exact measures employed to monitor it. We feel that dominance and submissiveness apply only in cases such that individual differences in aggressiveness have been "tested" by the antagonists and, to some extent, ritualized. Central to the latter point is a requirement that the establishment of a dominance hierarchy be accompanied by a reduction in the frequency of violent aggressive behaviors. On the basis of this criterion, it seems safe to conclude that most encounters described in this report occurred prior to and during (not after) the establishment of stable dominance orders. Flight occurred in only one of six female-female pairs. Further, other patterns of violent aggressive behavior such as Rolling Fights appeared regularly throughout the encounter series engaged in by nearly all pairs. Apparently, dominance relations within most pairs continued to receive active testing through very late stages in the encounter series, long after the establishment of correlations between following and the performance of On Back. We conclude that our descriptions of female hamster aggressive behaviors are relevant to the types of relatively prolonged agonistic interactions (excluding Flight) most likely to actually occur among wild individuals.

CHAPTER III. THE HORMONAL CONTROL OF FEMALE HAMSTER AGGRESSION

The fighting behavior of many species is sexually dimorphic, females generally exhibiting lower frequencies of aggressive behavior than males (Collias, 1944; Guhl, 1961). Superficially, this is consistent with the androgen-dependence of aggression in the males of many species (Chapter I) and the lower levels of androgens typically found in females. However, female golden hamsters (Mesocricetus auratus Waterhouse) are dramatic exceptions from the rule regarding female aggressive behavior. These animals generally are highly aggressive toward conspecifics of either sex (Kislak & Beach, 1955; Dieterlen, 1959; Payne & Swanson, 1970, 1971b-c and 1972c; Tiefer, 1970; Vandenbergh, 1971). Their agonistic behaviors are similar in form and intensity to those exhibited by males of the same species. In fact, female hamsters have been reported to dominate males in heterosexual encounters (Dieterlen, 1959; Payne & Swanson, 1970; Lerwill & Makings, 1971).

Reports conflict regarding the effects of various hormonal manipulations on the aggressive behavior of female hamsters. Ovariectomy has been associated with a slightly decreased incidence of female aggression in heterosexual (Kislak & Beach, 1955; Tiefer, 1970; Payne & Swanson, 1971b and 1972c) and unisexual (Payne & Swanson, 1971c) pairings. On the other hand, ovariectomized and intact individuals observed in female-female encounters by Vandenbergh (1971) exhibited similar levels of aggression.

Kislak and Beach (1955) reported a small increase in female aggressiveness during heterosexual encounters consequent to the administration of large doses (0.225 mg/day) of estradiol benzoate (EB). Lower doses of EB, or of testosterone propionate (TP) have not significantly altered the aggressivity or relative success of ovariectomized females encountering intact males (Payne & Swanson, 1971b and 1972c). Similarly, the aggressive behavior of gonadectomized females paired with intact females has proved resistant to change by EB or TP therapy (Tiefer, 1970; Payne & Swanson, 1971c and 1972c; Vandenbergh, 1971; also see Grelk, Papson, Cole & Rowe, 1974).

Progesterone (P) has been associated with increased levels of female hamster aggression in male-female (Payne & Swanson, 1971b and 1972c) and female-female (Payne & Swanson, 1971c and 1972c) encounters. At least in the former situation, the change in female dominance status consequent to the initiation of P therapy seems to have been incidental to a decrease in the intensity of male aggressive behavior. This suggests the operation of a progesterone-dependent cue limiting male aggression, rather than a direct effect of P upon female aggressivity (also see Payne, 1974). The increased aggressiveness exhibited by P-treated ovariectomized females paired with intact females suggests that P may exert both indirect and direct effects upon female aggressivity. Kislak and Beach (1955) however, have described the failure of relatively low doses of P to affect the aggressive behavior of two ovariectomized female hamsters in heterosexual pairings. Grelk et al. (1974) also have described the failure of a variety of progesterone dosages (100-1000 μg/day) to affect the aggressiveness of group-caged or individually-housed ovariectomized females.

The combination of EB and P has been associated with a profound decrement in female aggressivity in heterosexual pairings (Kislak & Beach, 1955; Tiefer, 1970). In female-female encounters, EB plus P also has tended to suppress fighting (Tiefer, 1970). While 22% of gonadectomized, untreated females initiated fights during tests with estrous females, no female treated with 6 μg EB and 0.4 mg P fought under the same circumstances.

The intense aggression exhibited by female hamsters is unusual among female vertebrates, and has stimulated much interest in its hormonal basis. However, results summarized above defy easy generalization. Difficulties may stem from the use of large doses of hormones, or to a common failure to eliminate all endogenous sources of sex steroids (gonads and adrenals). Furthermore, participants in many experiments were shuttled through long series of tests under several different hormone treatments, sometimes without appropriate controls for variations in aggressiveness with experience or age.

The degree of social contact experienced by experimental subjects, and its relation to the species' normal social organization, may be particularly important variables. Indirect evidence suggests strongly that free-ranging golden hamsters lead a relatively solitary existence (Chapter II). Yet, most previous studies of female hamster aggression have paired group-caged individuals in long social encounters, conditions which may have no natural analogues (e.g., Kislak & Beach, 1955; Payne & Swanson, 1970, 1971b-c and 1972c). In contrast, we feel that the initial phases of an encounter or encounter series, those preceding attempted escape by either individual, may be crucial in hamster social relations. Thus, the early stages of an encounter series involving naive individuals seems to provide the most interesting setting in which to investigate the hormonal bases for female hamster aggression (also see Brain, 1972b; Grelk et al., 1974; and, Wise, 1974). Accordingly, we have described the behaviors and sequences of behavior typical of early encounters between nonestrous female hamsters (Chapter II). The next step in this analysis is the examination of variations in female hamster aggression accompanying natural fluctuations in hormone state.

EXPERIMENT 2: CHANGES IN AGGRESSION DURING THE ESTROUS CYCLE

Method

Subjects, Maintenance and Experimental Pairings

Twelve random-bred female hamsters of the LVG:LAK strain were purchased from Lakeview Hamster Colony, Newfield, N.J. at 28-35 days of age (shortly after weaning). These were the same subjects and the same experimental encounters as described previously in a detailed account of the behavioral elements and sequences characteristic of fights among female hamsters (Chapter II). Thus, conditions of maintenance and the compositions of experimental pairs were as previously described.

Test Schedules and Procedures

Each of six pairs of intact adult female hamsters experienced a series of eight 3-minute encounters in a clean neutral arena. The time of day of testing was held constant (± 30 min.) for individual pairs and ranged between 3.5 and 6.5 hours after the start of the dim-light segment of the diurnal cycle. Tests were scheduled so that each pair experienced only one encounter per test-day. Successive tests typically were conducted at 3-day intervals, until each pair had experienced two encounters on each of the four days of the normal estrous cycle. Different pairs began the sequence of eight regularly spaced tests at different stages of the estrous cycle. Procedures involved in conducting experimental social encounters have been described (Chapter II).

Behavioral Categories and Analysis

Agonistic behaviors were scored during the frame-by-frame analysis of movies viewed on a small screen, using a modified Honeywell projector (Lafayette Super 8 Analyzer). The measures employed in analyses of the agonistic behavior of experimental females included the latency and total duration of each of the following behaviors: Upright, On Back, Rolling Fight, Fly-Away and Lordosis. The latency of Flight and an estimate of the frequency of Biting (the percentage of 20-frame (11-second) blocks in which at least one bite occurred) also were tabulated. The latencies and durations of behaviors were determined as previously described (Chapter II). Experimental encounters continued for 3 minutes, or until the initial appearance of Flight. Therefore, the maximum latency achievable by any behavior was 180 seconds.

Statistics

For most statistical analyses, scores contributed by a single experimental pair on two comparable days of the estrous cycle (e.g., the two tests on estrous day, or "Day 1" of the four-day cycle) have been averaged. Median latencies and mean durations for the group of six pairs have been calculated for each behavioral measure. Average scores at different stages of the estrous cycle have been compared on the basis of sign-tests (Siegel, 1956) and t-tests for dependent means

(Edwards, 1966). In each case, the counterbalanced order (with respect to cycle day) in which pairs were tested has allowed each experimental pair to serve as its own control. All statements regarding the statistical significance or chance probabilities of observed differences have been based on two-tailed tests of significance.

Results

Latencies of all agonistic behaviors considered indicate that levels of female-female aggression were lower on estrous day ("Day 1") than on any of the other three days of the cycle (Figure 8). For On Back and Rolling Fight, median latencies on estrous day are significantly longer than those on Days 2, 3 or 4 ($p < .05$, sign-test). This reflects a high degree of consistency among the six pairs of females (Figures 9-10).

On Back and Rolling Fight, both moderate to high intensity aggressive responses according to our previous description (Chapter II), exhibited the most pronounced cyclic variations in aggressiveness (Figures 8-10). A more preliminary behavior, Upright, tended to occur in a high percentage of encounters with low latencies (Figure 8). Conversely, the most intense responses, Fly-Away and Flight, rarely occurred, and consequently have average latencies close to 180 seconds throughout the estrous cycle (Figure 8).

Under our conditions of testing and analysis, latencies of Biting varied throughout the estrous cycle (Figure 8). To some extent, this may reflect our relative inability to reliably discriminate Bites in super-8 films of encounters. In addition, however, estrous female rodents sometimes are observed to bite gently the skin of inactive males, and this, too, could account for the relatively high frequencies of Biting by estrous female hamsters in these encounters. The development of severe Biting, associated with high levels of aggressivity, may require particular responses on the part of the bitten individual to initial oral contact concerned with social investigation or sexual arousal as well as aggression.

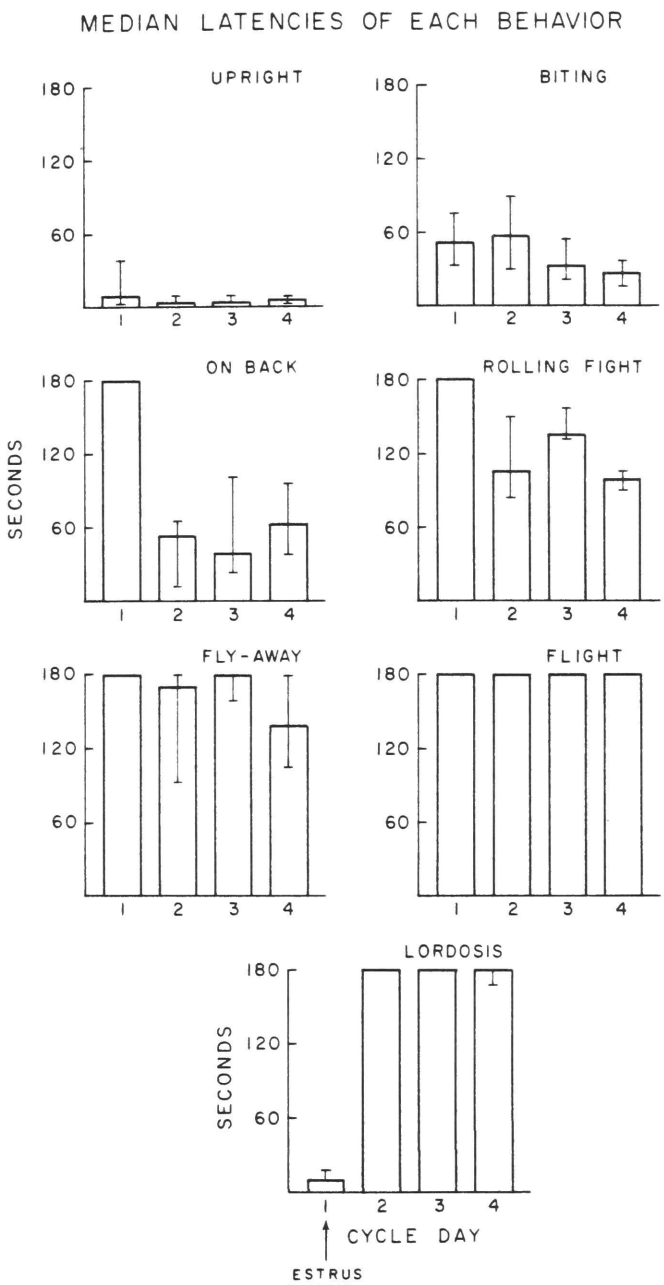


Figure 8. Median latencies of 7 behavior patterns during the normal 4-day estrous cycle. Each histogram indicates the median latency for 12 encounters (6 intact female-female pairs) on a particular day of the cycle. Latencies corresponding to the first and third quartiles are specified, thus indicating the interquartile range of each distribution (see Edwards, 1966). Estrous day is designated Day 1, and nonestrous days are Days 2-4. The maximum latency attainable is 180 seconds. Latencies of ON BACK, ROLLING FIGHT and LORDOSIS on Day 1 differ significantly from those on Days 2-4.

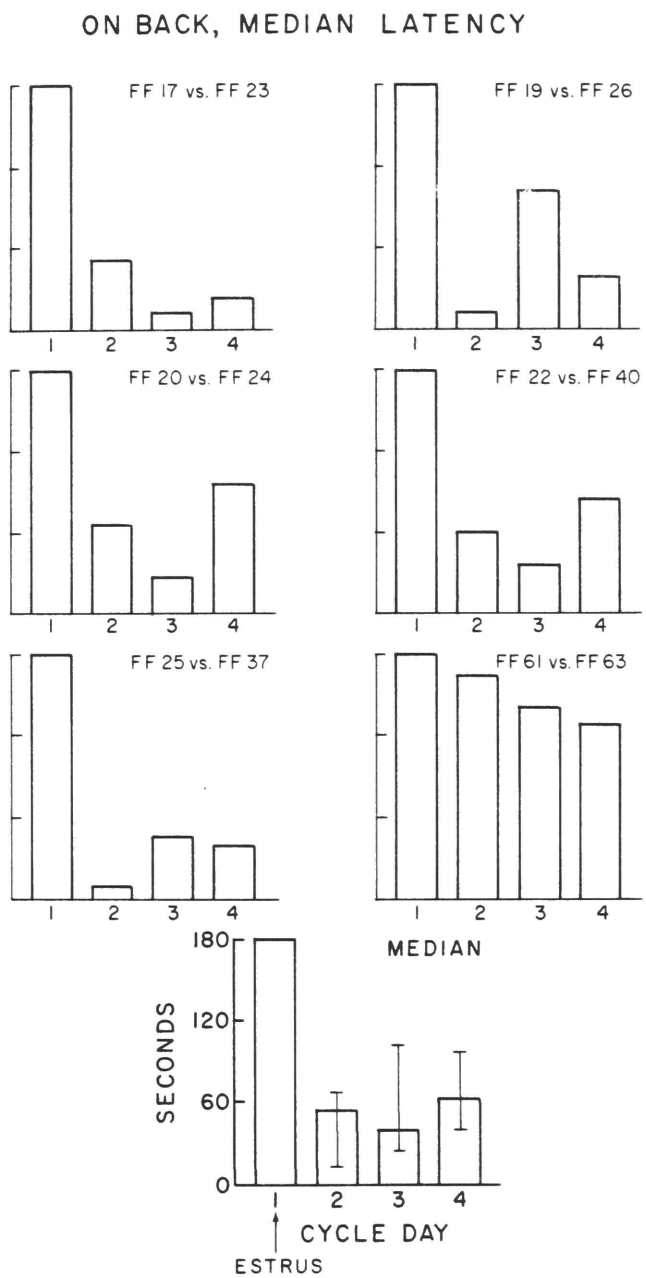


Figure 9. Median latencies of ON BACK during the normal 4-day estrous cycle. Each of 6 pairs of intact female hamsters (e.g., FF17 vs. FF23) experienced a series of eight 3-minute encounters (2 on each of the 4 cycle days). Median latencies compiled by each pair are summarized, as well as the median across pairs (bottom). For the latter, latencies corresponding to the first and third quartiles are specified, thus indicating the interquartile range of each distribution. Estrous day is designated Day 1, and nonestrous days are Days 2-4. The maximum latency attainable is 180 seconds. Differences between Day 1 and each of Days 2-4 are statistically significant.

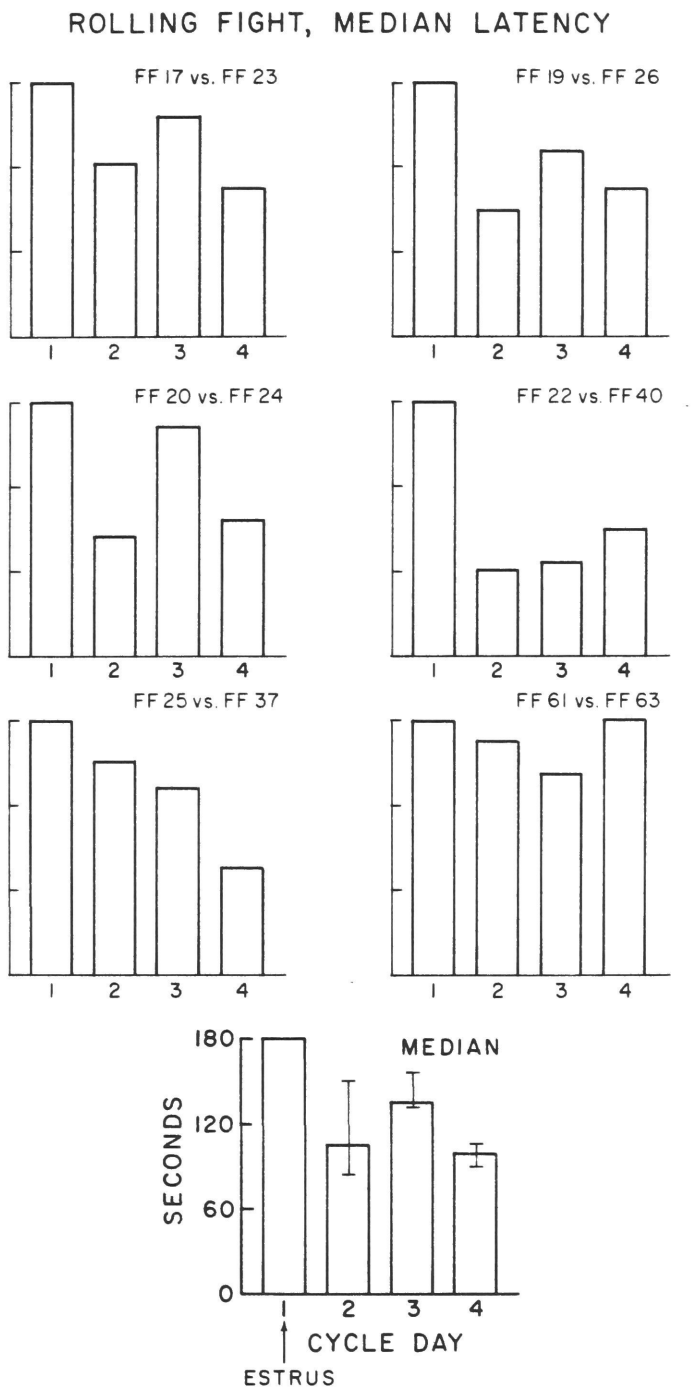


Figure 10. Median latencies of ROLLING FIGHT during the 4-day estrous cycle. See legend of Figure 9 for details. Differences between Day 1 and each of Days 2-4 are statistically significant.

In contrast to agonistic behaviors, which occurred less frequently and with longer latencies on estrous day, Lordosis occurred with minimal latency on estrous day (Figure 8). Thus, as is the case in heterosexual encounters (Kislak & Beach, 1955; Payne & Swanson, 1970; Tiefer, 1970; and, Wise, 1974), increased sexual receptivity on estrous day is associated with a profound decrease in the likelihood and severity of aggressive behavior (also see Figure 15).

The mean durations of moderate to high intensity female-female agonistic behaviors lead to similar conclusions. Agonistic behaviors tended to have shorter durations on estrous day (Figure 11). These differences are statistically significant ($p < .05$, sign test) for On Back and Rolling Fight. For these behaviors, the cyclic variation is highly consistent across pairs of females, even though pairs differed markedly in basal (nonestrous) durations of these aggressive responses (Figures 12-13). In contrast, durations of Lordosis were significantly longer ($p < .05$, sign test) on estrous days (Figures 11 and 14). Neither the median latencies nor the mean durations of any agonistic behavior varied significantly within the nonestrous phase of the estrous cycle (Days 2-4).

We have shown that significant variations in agonistic behavior sequences occurred during the series of eight brief social encounters experienced by these female-female pairs (Chapter II). Thus, it is important to note that trends summarized above are not highly specific to particular stages of the encounter series. For example, average latencies and durations of On Back observed on estrous days differed significantly from those on the first through sixth nonestrous tests considered individually ($p < .05$ for all comparisons, sign test). Similarly, average latencies and durations of Rolling Fights on estrous days differed from those on the third, fourth and fifth nonestrous tests considered individually ($p < .05$, sign test). In this regard, the propensity of a pair of female hamsters to exhibit intense aggressive responses varies with social experience, and the pattern of variation depends on the particular behavior pattern considered. In the case of On Back, such variations are relatively minor. The first through sixth nonestrous encounters in these tests series were associated with

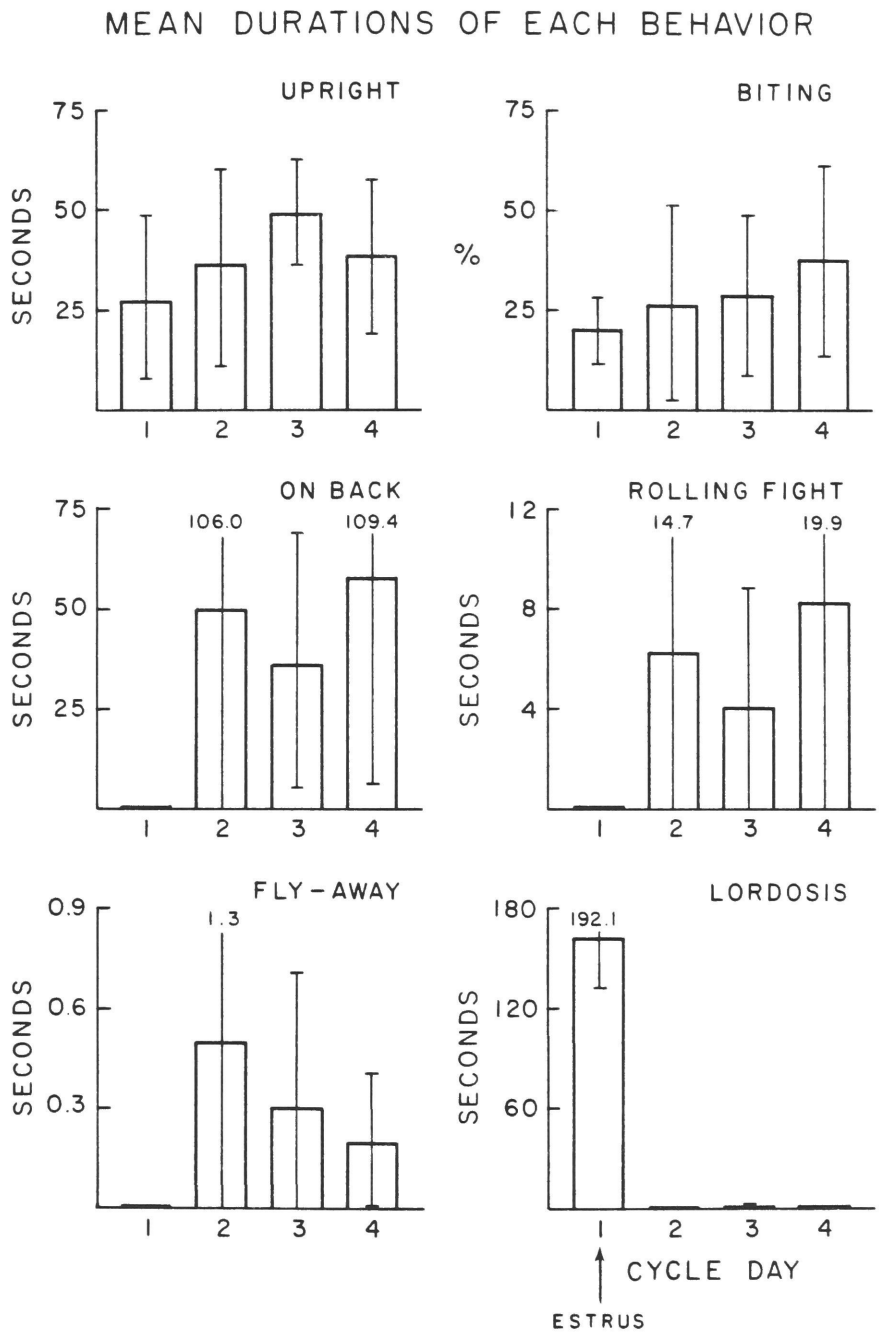


Figure 11. Mean durations of 6 behavior patterns during the normal 4-day estrous cycle. Each histogram summarizes the mean duration (± one standard deviation) observed in 12 encounters (6 intact female-female pairs) on a particular day of the cycle. Estrous day is designated Day 1, and nonestrous days are Days 2-4. Mean durations of ON BACK, ROLLING FIGHT and LORDOSIS on Day 1 differ significantly from those on Days 2-4.

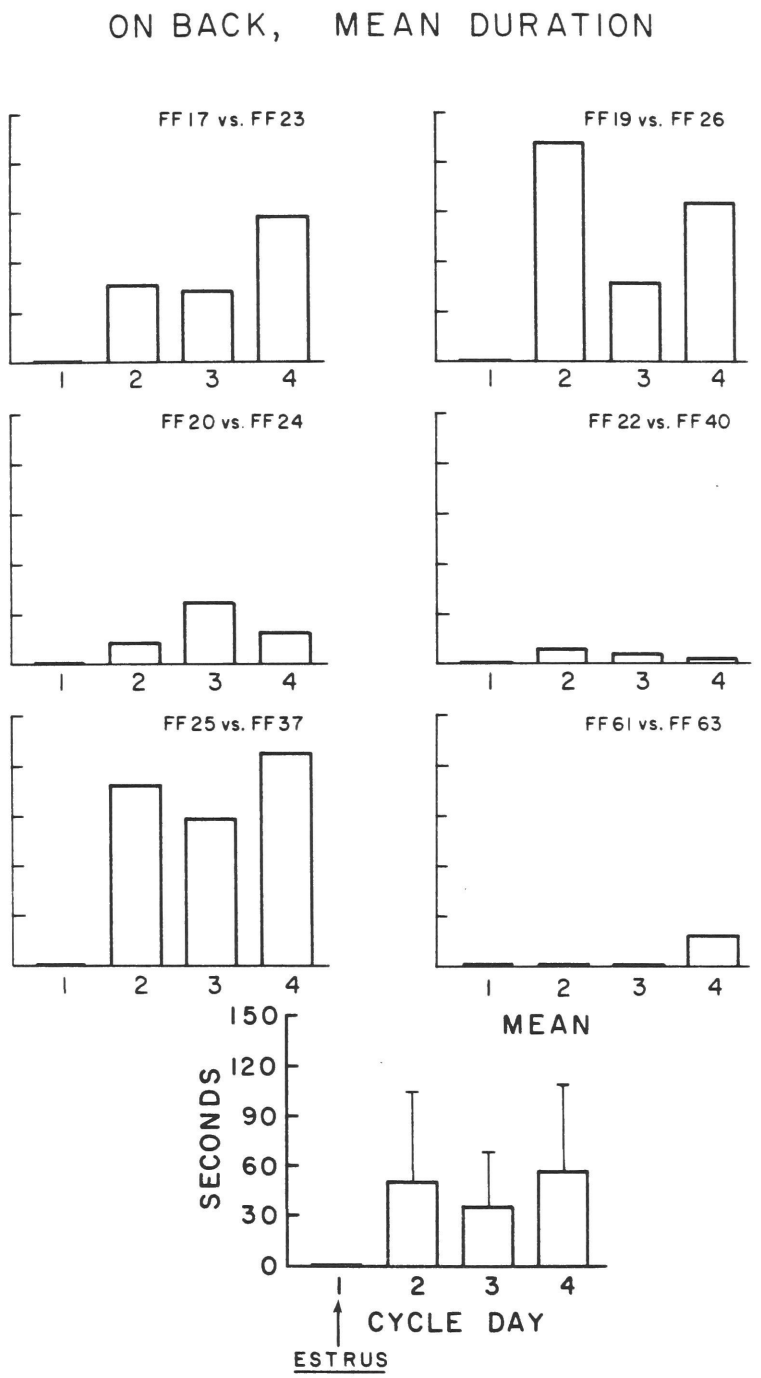


Figure 12. Mean durations of ON BACK during the normal 4-day estrous cycle. Each of 6 pairs of intact female hamsters (e.g., FF17 vs. FF23) experienced a series of eight 3-minute encounters (2 on each of the 4 cycle days). Mean durations compiled by each pair are summarized as well as the mean (+ 1 S.D.) across pairs (bottom). Estrous day is designated Day 1, and nonestrous days are Days 2-4. Differences between Day 1 and each of Days 2-4 are statistically significant.

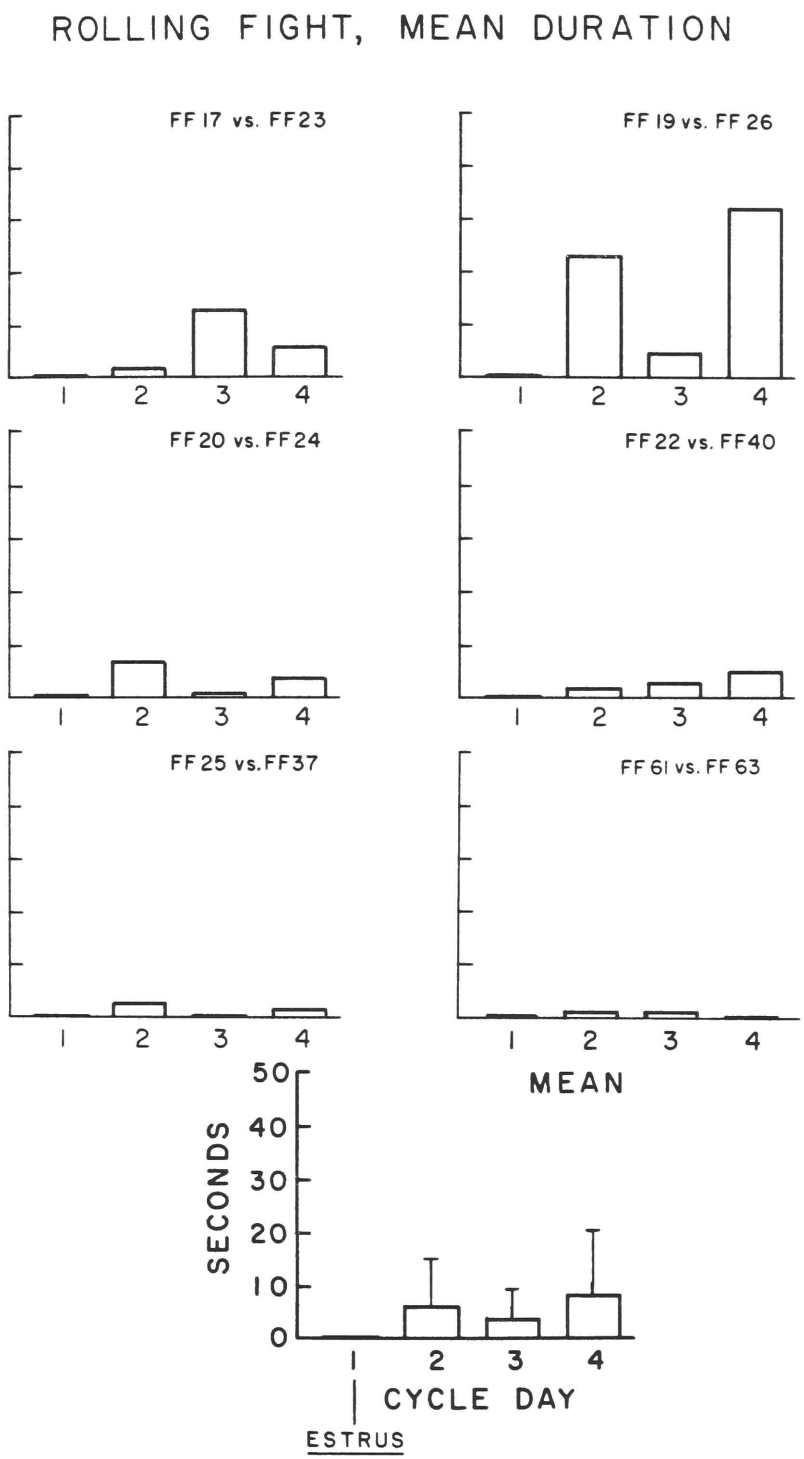


Figure 13. Mean durations of ROLLING FIGHT during the normal 4-day estrous cycle. See legend of Figure 12 for details. Differences between Day 1 and each of Days 2-4 are statistically significant.

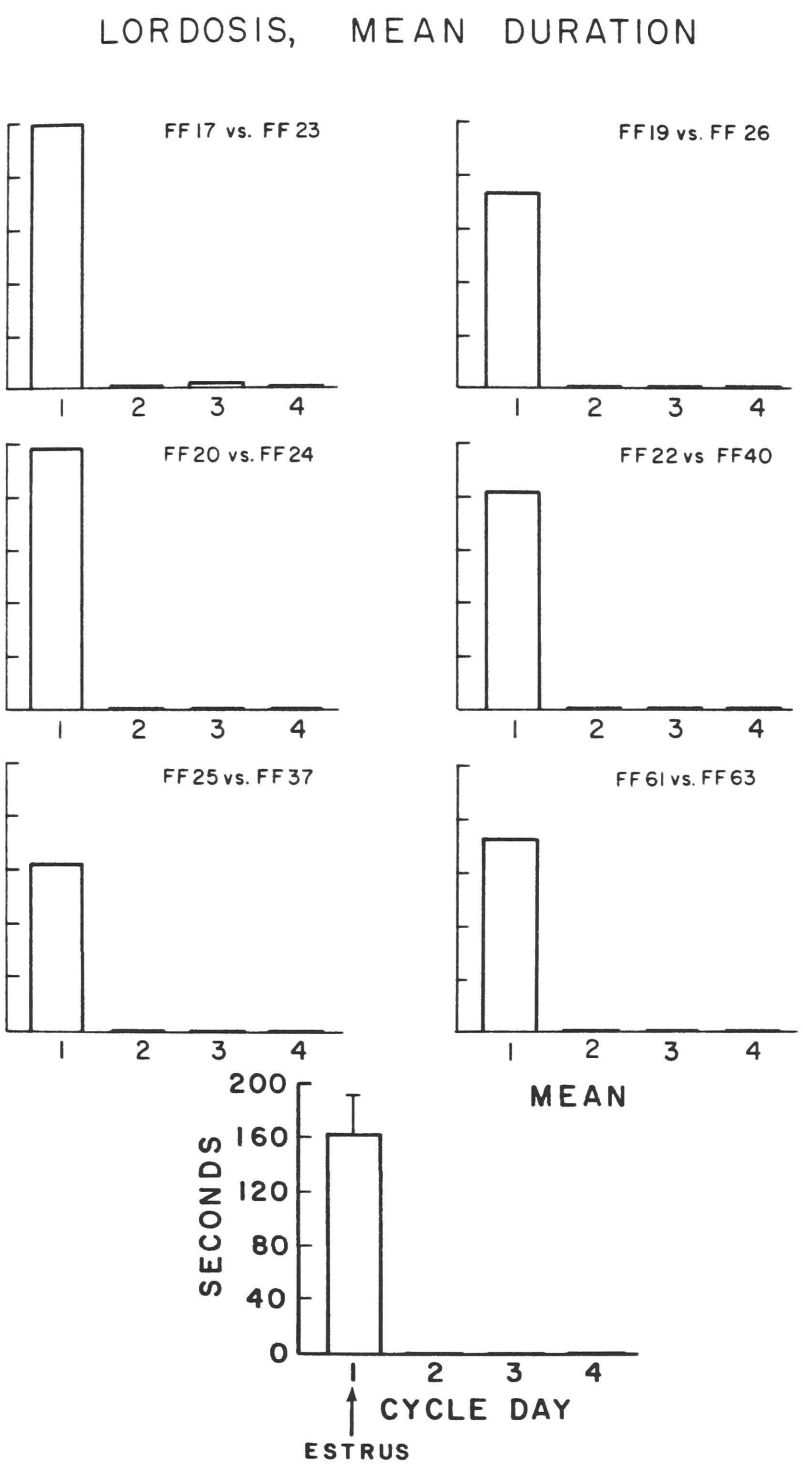


Figure 14. Mean durations of LORDOSIS during the normal 4-day estrous cycle. See legend of Figure 12 for details. Differences between Day 1 and each of Days 2-4 are statistically significant.

similar On Back latencies and durations. Accordingly, On Back postures were exhibited less frequently on estrous days than on any nonestrous day regardless of placement within the test series. On the other hand, Rolling Fights are most prepotent during intermediate encounters in the series of six nonestrous tests (see Figures 5-7). For example, Rolling Fight durations on the third, fourth, and fifth nonestrous tests consistently exceeded those seen in the first nonestrous encounter ($p < .05$, sign test). One consequence of such variations is the restriction of consistent differences in Rolling Fight latencies and durations to comparisons of estrous day encounters with interactions during the 3rd - 5th nonestrous encounters.

Disucssion

The aggressive behavior exhibited by intact female hamsters varies dramatically as a function of the 4-day estrous cycle. The intensity of fighting is high throughout the nonestrous phase, without significant differences among nonestrous days. In contrast, on estrous day (Day 1), intense aggression is virtually absent.

Variations in aggressiveness seen in pairs composed of two synchronously cycling females are identical to those observed during pairings of males with intact females (Figure 15; see also, Kislak & Beach, 1955; Payne & Swanson, 1970; Tiefer, 1970; Wise, 1974). Further, these data are in complete accord with suggestions by Tiefer (1970) and Vandenbergh (1971) regarding cyclic variations in the intensity of aggression exhibited by intact female-female pairs. Under natural conditions, the coincidence of ovulation and lordosis with the suppression of aggressive behavior clearly is biologically adpative, since it facilitates male-female contact at the only point during the female's cycle at which successful mating can occur.

Variations in aggressiveness during the estrous cycle of the intact female hamster may be related to our description of the sequences of aggressive behavior characteristic of this animal (Chapter II). Preliminary aggressive responses, such as the Upright posture, occur with relatively short latencies in a high percentage of encounters throughout the estrous cycle. Responses corresponding to the most extreme forms

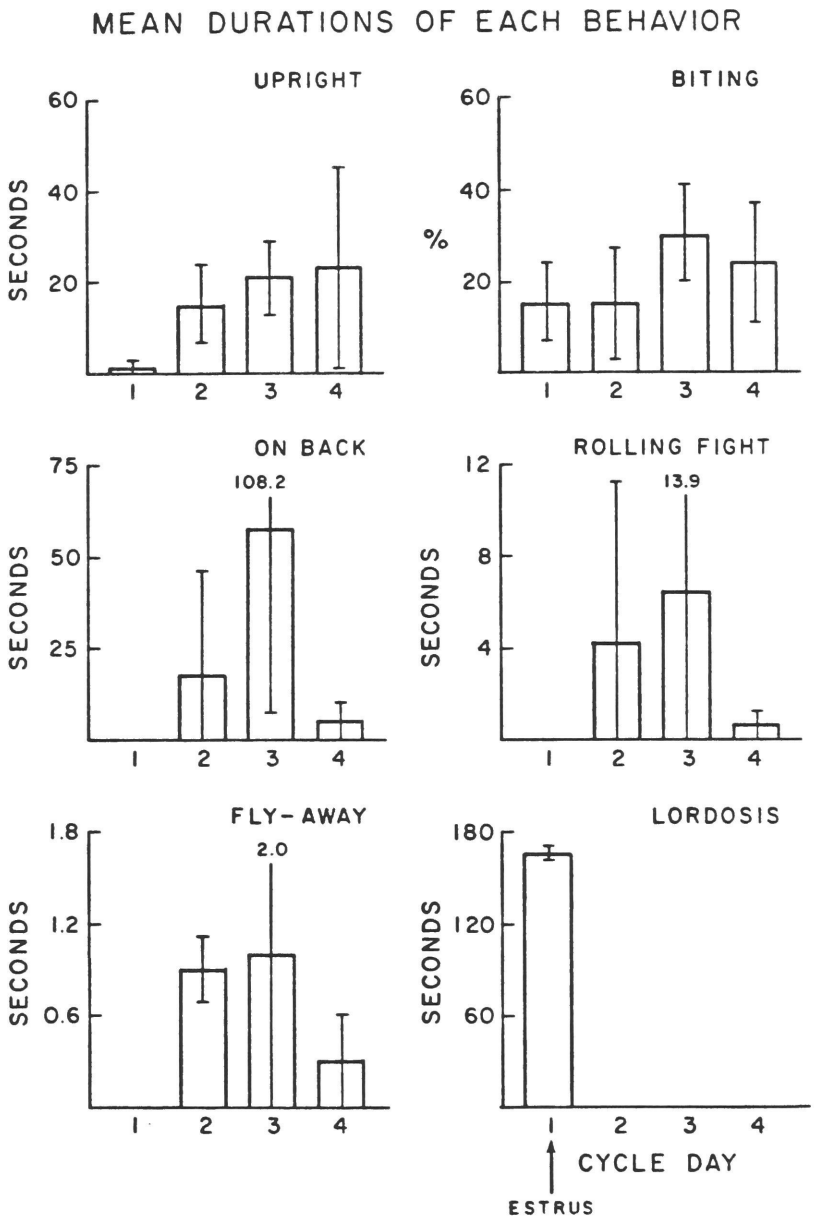


Figure 15. Aggressive responses during male-female interactions. Each of 4 intact adult males was paired with a female of similar body weight. Each pair experienced a series of 4 encounters at 3-day intervals, one encounter on each of the 4 days of the female's estrous cycle (all females were intact and maintained regular 4-day estrous cycles throughout testing). Different pairs began the series of 4 tests at different stages of the female's cycle. Encounters were staged and scored as described in the text for female-female encounters. In this figure, mean durations (± one standard deviation) of 6 behavior patterns are displayed as functions of the 4-day estrous cycle of the female participant. Day 1 refers to the estrous day of the female partner. Days 2-4 are her nonestrous days. Note the similarity between trends described here and those summarized in Figures 8-14.

of aggressiveness, such as Fly-Away and Flight, must be preceded by a long chain of less intense aggressive responses, and occur with very long latencies or do not occur at all. Hormonal variations occurring during the estrous cycle have their most pronounced effects on moderate or high intensity aggressive responses such as the On Back posture and Rolling Fight. The failure of the estrous female hamster to exhibit these aggressive responses quickly or often may reflect a failure of the estrous animal to Attack (or to respond aggressively to an Attack by) its opponent.

EXPERIMENT 3: AGGRESSION IN ADRENALECTOMIZED-OVARIECTOMIZED FEMALES

The suppression of aggression on estrous day coincides with the emergence of lordosis as a highly prepotent response. Since a combination of estradiol and progesterone is required for the induction of lordosis in ovariectomized female hamsters (Ciaccio & Lisk, 1971), either or both of these hormones may be responsible for the inhibition of aggression typical of estrous day (see Kislak & Beach, 1955; Tiefer, 1970; and, Payne & Swanson, 1971b). Plasma levels of both hormones typically exhibit sharp increases to maximal levels before the beginning of the lights-out period during which ovulation occurs (Baranczuk & Greenwald, 1973; Lukaszewska & Greenwald, 1970).

Hormones also could be necessary for the induction of vigorous aggression in the female hamster during nonestrous phases of her cycle (see Kislak & Beach, 1955; Payne & Swanson, 1971b-c and 1972c). For example, the major fluctuations in peripheral levels of estrone adhere to a diurnal cycle, consistently reaching peak values near the midpoint of the dark phase of the day (Baranczuk & Greenwald, 1973). Any variation with the estrous cycle here is much less pronounced than in the cases of estradiol or progesterone. Further, observations by Lerwill and Makings (1971) suggest that male hamster aggressiveness varies according to a diurnal cycle, and the pattern of fluctuation resembles that in peripheral levels of estrone. Alternatively, Payne and Swanson (1971b and c, 1972c) have implicated progesterone in the maintenance of high levels of female aggression in both female-female and male-female social interactions.

Method

Subjects, Maintenance and Surgical Manipulation

Twenty-eight random-bred female hamsters of the LVG:LAK strain were purchased from Lakeview Hamster Colony at 28-35 days of age (shortly after weaning). Females were caged individually and maintained as previously described. Estrous cycles were monitored (see Orsini, 1961), and females required to exhibit at least four successive 4-day cycles before surgical intervention at 123-352 days of age. Bilateral adrenalectomy and ovariectomy was performed under Equithesin anesthesia (Jensen-Salsbery Laboratories, Kansas City, Missouri, .35cc/100 gm). Routine maintenance of adrex-ovariex individuals included a single injection of 1.25 mg of a long-lasting mineralocorticoid preparation, desoxycorticosterone pivalate (Percorten pivalate, CIBA Pharmaceutical Company, Summit, New Jersey) two days before surgery. Desoxycorticosterone (DOC) replacement therapy was necessary for the continued survival of adrex-ovariex hamsters, and was administered in 0.75 mg doses at 10-14 day intervals throughout the period of experimentation. Operated females also were supplied frequently with cabbage, and had continuous access to an aqueous solution of NaCl (0.9% or 2%), dextrose or sucrose (2%) and terramycin (.03%), as well as tap water.

At least 19 days (range = 19-73 days) were allowed for recovery from operative procedures. Individuals ranged between 148 and 397 days of age at the initiation of testing. Most (79%) tests were conducted during July-August to avoid possible seasonal variations in aggressiveness.

Hormones and Test Schedules

Adrex-ovariex female hamsters were tested under the following regimes of hormone treatment: Testosterone propionate (TP, 200 µg/day, 4 pairs); Estradiol benzoate (EB, 10 µg/day, 4 pairs); Progesterone (P, 500 µg/day, 3 pairs); Estradiol benzoate plus progesterone (EB + P, 10 µg/day of EB plus a single injection of 500 µg P approximately four hours prior to testing, 4 pairs); or, oil vehicle alone (3 pairs). EB and TP were purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio, while P was purchased from Matheson Coleman and Bell,

East Rutherford, New Jersey. All hormone dosages were dissolved in 0.1 ml of sesame oil (purchased from Amend Drug and Chemical Co., Inc., New York, New York) and were administered subcutaneously approximately 22 hours prior to testing. Tests were scheduled at 3-4 day intervals, and the first day of testing coincided with the eighth day of daily hormone treatment. Both members of a particular pair were similar in body-weight, and were subjected to identical hormone treatments. Most hormone-treatment groups were independent in that most pairs of females experienced only a single series of four encounters under one hormone. Groups treated with EB represent the only exception. Here, a single EB + P test was inserted at the midpoint of a series of four EB tests.

Procedures, Behavioral Categories and Analysis

The procedures involved in conducting experimental social encounters, and the categories of social behavior scored from movies of these encounters, have been described in a previous chapter. Methods of analysis, and the particular categories of agonistic behavior employed here as measures of female aggression, have been specified in a previous section of this chapter.

Verification of Surgical Procedures

Following the completion of aggression-testing, DOC replacement therapy was discontinued and the body-weights of experimental subjects monitored over the next 1-2 months. Most females (26 of 28) suffered precipitous losses of weight, or death, within this period, consistent with complete adrenalectomy. The two surviving females were autopsied and found to have small fragments of tissue which, in terms of gross morphology and location, resembled normal adrenal tissue. Records contributed by these females in aggression testing did not differ consistently from other members of their respective hormone-treatment groups. Thus, data contributed by these females have been included in the summary below.

Statistics

Median latencies, and mean durations, contributed by different hormone-treatment groups were compared on the basis of the Fisher exact probability test (Siegel, 1956). Mean durations also were evaluated using the t-test for independent means (Edwards, 1966).

Results

Adrex-ovariex female hamsters receiving only oil injections fought at levels comparable to those exhibited by intact, nonestrous females, and considerably above levels of aggressivity characteristic of intact estrous females (Figures 16-19). Replacement therapy with TP, P or EB individually did not exert consistent significant effects upon the aggressiveness of these females (Figures 16-19). Adrex-ovariex females receiving 200 µg/day of TP required significantly longer latencies for the appearance of On Back postures than did intact nonestrous females ($p < .05$, Fisher test). However, these females clearly did engage in some violent fights (e.g., Figure 19) and could not be distinguished reliably from intact nonestrous females, or adrex-ovariex oil controls, on the basis of any measure other than On Back latency. These conclusions are not highly dependent on the amount of prior social experience: individual comparisons of the first or fourth encounters in the series experienced by hormonally manipulated and intact groups gave similar results.

In contrast to the magnitude of hormonal effects observed under any other condition, the combination of EB and P exerted a profound suppressive effect upon female aggressivity (e.g., mean durations of On Back, Rolling Fight and Lordosis associated with control oil treatment differed significantly from mean durations during EB + P therapy, $p < .062$, t-test: also, see Figures 16-20). In fact, levels of aggressive (Figures 16-19) and sexual (Figure 20) behaviors appearing under the influence of EB plus P closely resembled those exhibited by intact estrous females and consistently differed from those characteristic of intact nonestrous females (e.g., mean durations of On Back, Rolling Fight and Lordosis associated with EB + P treatment differed significantly from mean durations compiled by intact nonestrous females in Experiment 1, $p \leq .05$, Fisher test).

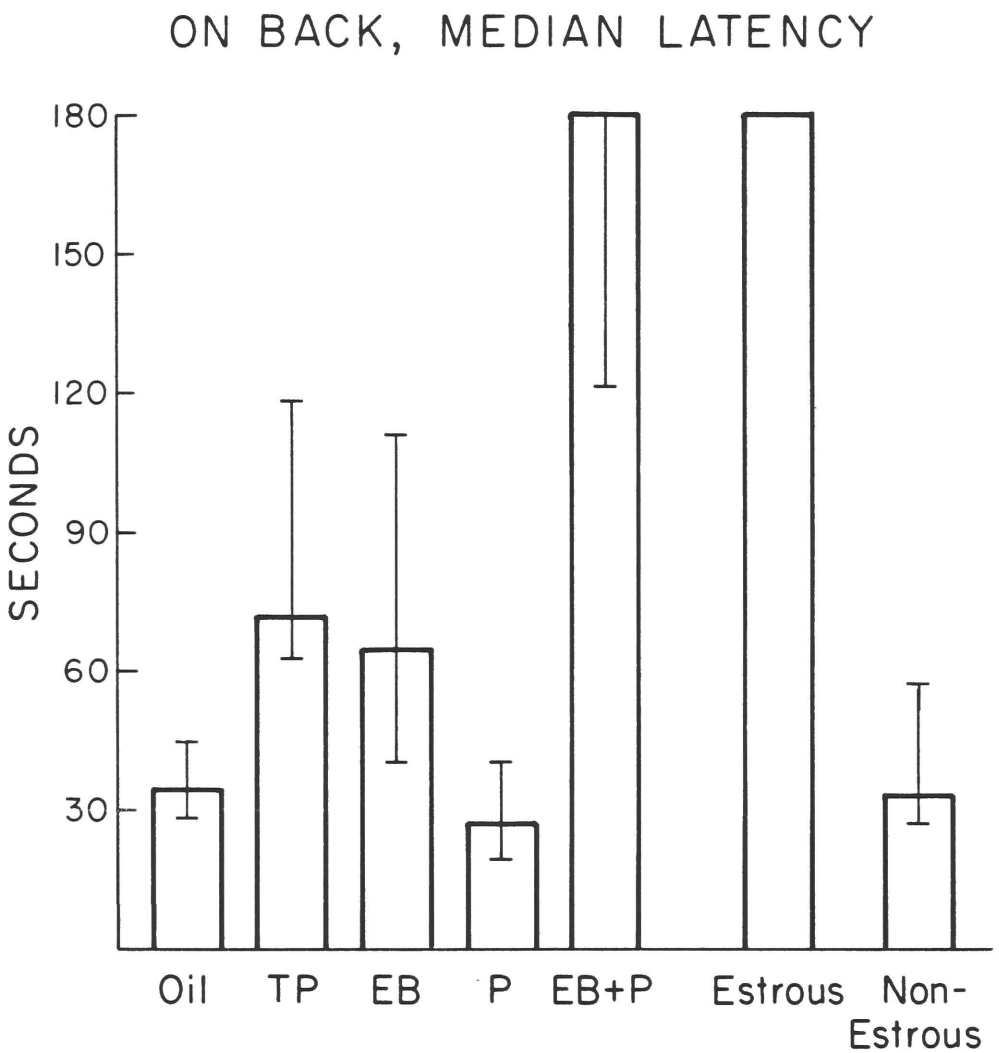


Figure 16. Effects of exogenous steroids on the median latency of ON BACK. Each of fourteen pairs of adrenalectomized-ovariectomized female hamsters experienced a series of four 3-minute encounters under one of the following hormone regimes: testosterone propionate (TP, 200 μg/day); estradiol benzoate (EB, 10 μg/day); progesterone (P, 500 μg/day); or, control oil injections. EB plus P tests (EB + P) were inserted into each EB series; daily EB treatment 10 μg/day) was supplemented by a single P injection (500 μg) approximately 4 hours prior to testing. Median latencies of behavior on Estrous (Day 1) and Non-Estrous (Days 2-4) days of the normal estrous cycle are drawn from a previous experiment (see Figs. 8-10). Latencies corresponding to the first and third quartiles are specified, thus indicating the interquartile range of each distribution. According to this analysis, the combination of estradiol and progesterone reduced fighting to levels typical of normal estrus.

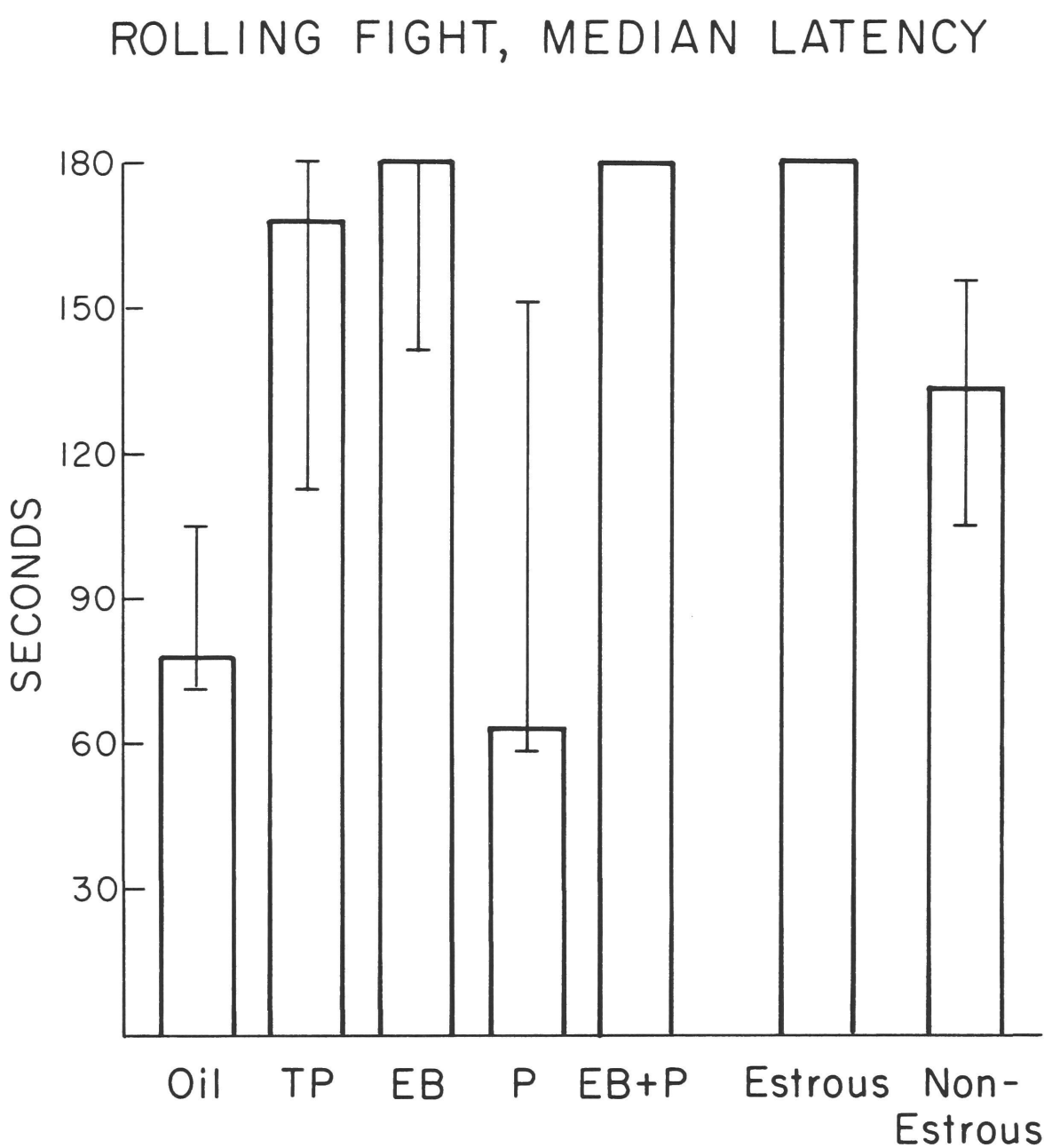


Figure 17. Effects of exogenous steroids on median latency of ROLLING FIGHT. See legend of Figure 16 for details.

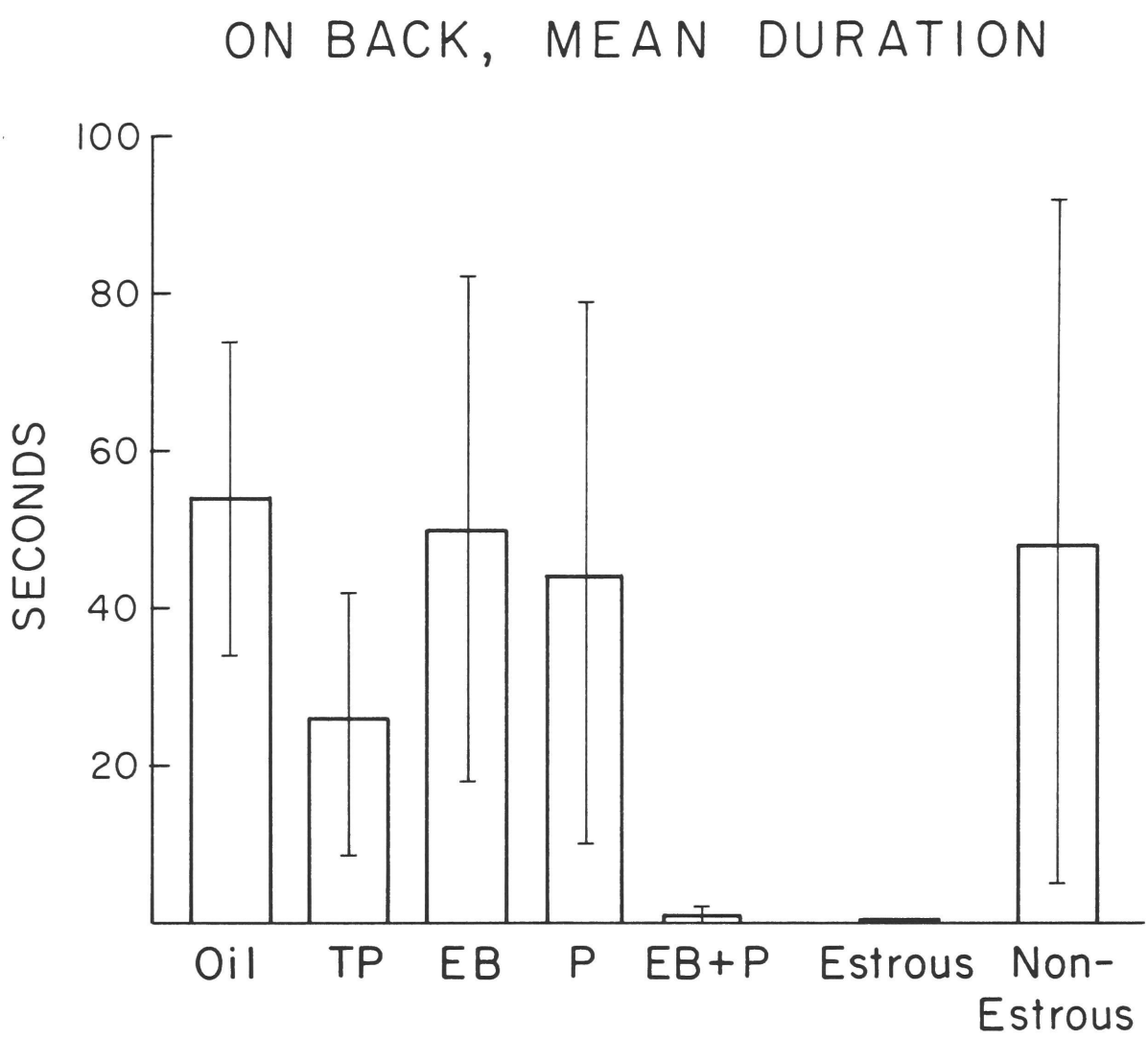


Figure 18. Effects of exogenous steroids on the mean duration of ON BACK. Each of fourteen pairs of adrenalectomized-ovariectomized female hamsters experienced a series of four 3-minute encounters under one of the following hormone regimes: testosterone propionate (TP, 200 μg/day); estradiol benzoate (EB, 10 μg/day); progesterone (P, 500 μg/day); or, control oil injections. EB plus P tests (EB + P) were inserted into each EB series; daily EB treatment (10 μg/day) was supplemented by a single P injection (500 μg) approximately 4 hours prior to testing. Mean durations (+ 1 S.D.) under these regimes may be compared directly with those on Estrous (Day 1) and Non-Estrous (Days 2-4) days of the normal estrous cycle (also see Figs. 11-14). Based on this analysis, the combination of estradiol and progesterone reduced fighting to levels typical of normal estrus.

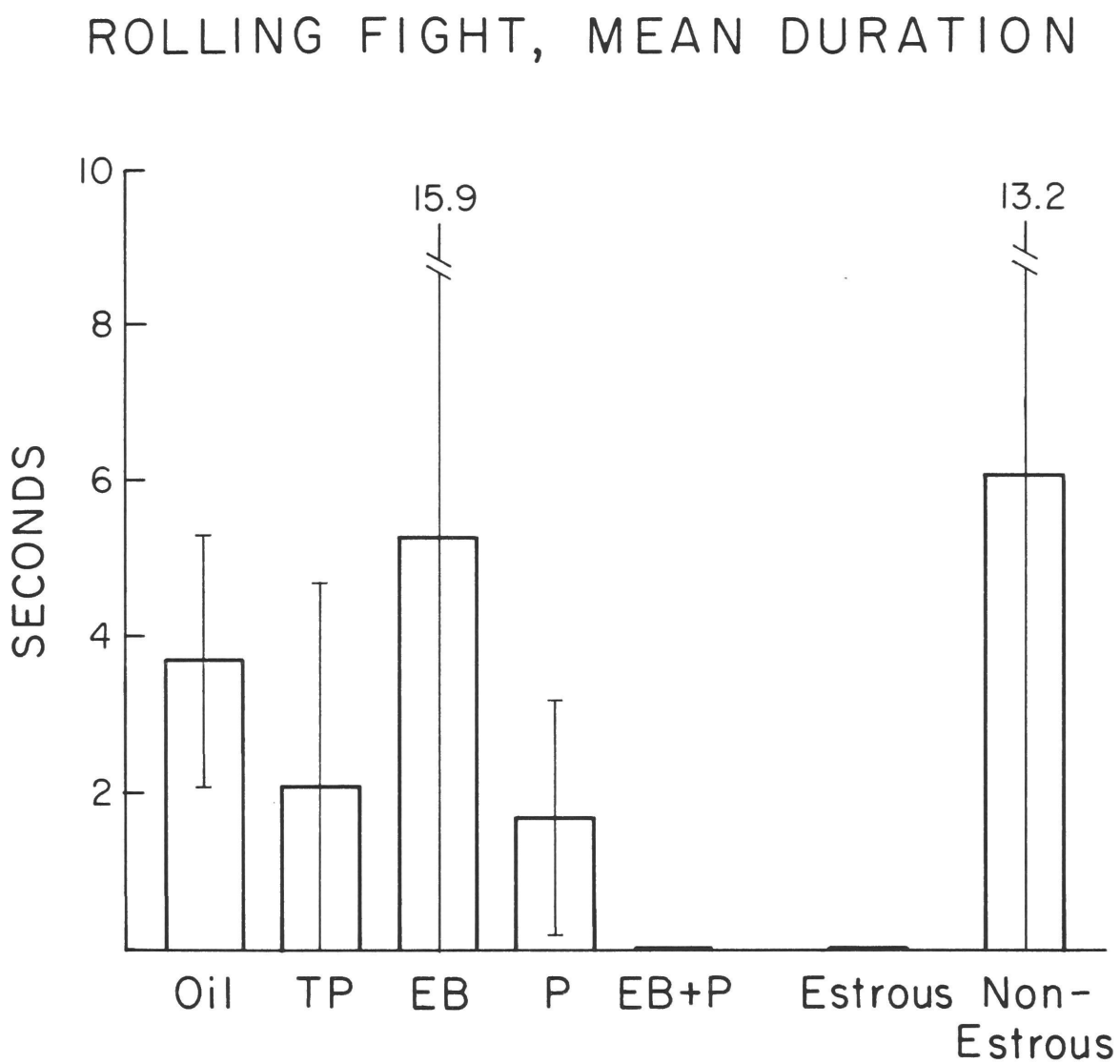


Figure 19. Effects of exogenous steroids on the mean duration of ROLLING FIGHT. See legend of Figure 18 for details.

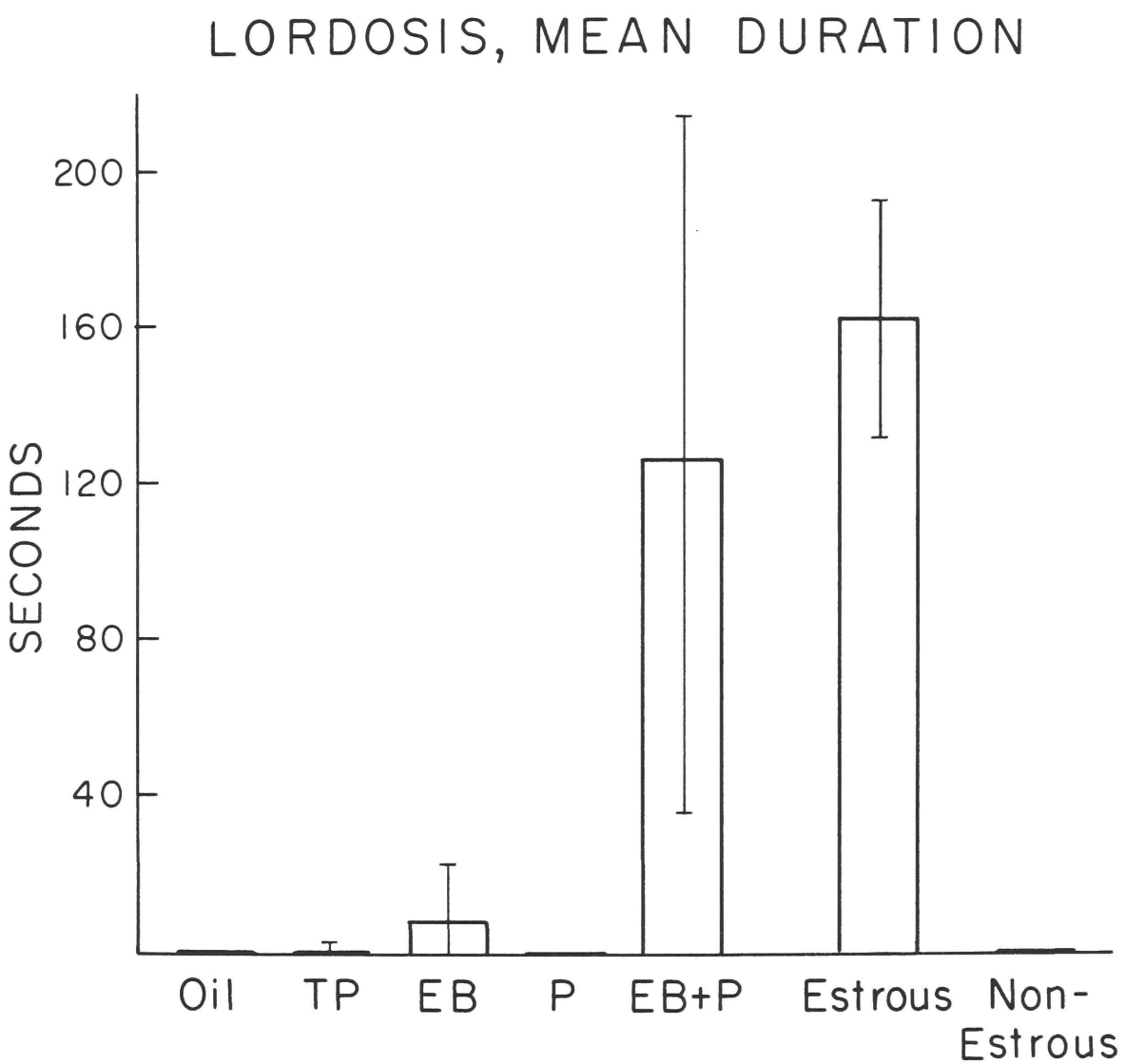


Figure 20. Effects of exogenous steroids on the mean duration of LORDOSIS. See legend of Figure 18 for details.

Discussion

Observations of aggressive behavior in adrex-ovariex females were addressed to the issue of the hormonal basis for cyclic fluctuations in female hamster aggressiveness. Adrex-ovariex females receiving only control oil injections exhibited high levels of aggression, comparable to those seen among intact nonestrous females. Administration of TP alone, or EB alone, or P alone had no consistent or dramatic effect on the aggressiveness of adrex-ovariex females. Levels of fighting remained high, comparable to those in intact nonestrous females and considerably above those characteristic of intact estrous females. These results suggest strongly that sex steroids cannot play more than a very minor role in the stimulation of vigorous aggressive behavior during non-receptive phases of the normal hamster estrous cycle. Indeed, estrogens and progesterone do not appear in relatively high peripheral concentrations throughout the nonestrous period, when levels of aggression are consistently high (see Baranczuk & Greenwald, 1973; and, Lukaszewska & Greenwald, 1970).

In marked contrast to effects of EB or P individually, the combination of these two hormones suppressed fighting completely, creating a situation virtually identical to that typical of estrous day. Thus, as suggested initially by Kislak and Beach (1955), it seems very likely that some combination of endogenous estrogen and progesterone provides the endocrinological basis for the inhibition of aggression exhibited by the estrous female hamster. Peak plasma levels of estradiol (Baranczuk & Greenwald, 1973) and progesterone (Lukaszewska & Greenwald, 1970) are achieved near the beginning of the dim-light period during which ovulation occurs, and during which aggression is inhibited and lordosis facilitated. This relationship seems highly adaptive. The mutual dependence of both aggression and sexual receptivity on the same hormonal combination (EB + P) would insure the most exact synchronization of these behaviors with each other and with endocrine (reproductive) state (see Experiment 1 of this chapter; also see, Kislak & Beach, 1955; Payne & Swanson, 1970; Tiefer, 1970; and, Wise, 1974).

Results summarized here are consistent with previous research with respect to the insensitivity of female hamster aggressive behavior to EB or TP individually, and regarding the inhibition of female hamster fights by the combination of EB and P (Payne & Swanson, 1971c and 1972c; Vandenbergh, 1971; also see, Kislak & Beach, 1955; Tiefer, 1970; Payne & Swanson, 1971b; and, Grelk et al., 1974). Further, these results are consistent with Vandenbergh's (1971) demonstration of the insensitivity of female hamster aggression to ovariectomy. In contrast, Payne and Swanson (1971c and 1972c) concluded that female hamster aggressiveness declines with ovariectomy and requires P replacement therapy for full restoration. We have uncovered no evidence for any such P-dependent facilitation of fighting in hamsters (also see Grelk et al., 1974).

Numerous methodological differences distinguish among these studies (e.g., strain of experimental subjects, hormone dosages, operative procedures, measures of aggression). One of the most conspicuous concerns the extent of social contact experienced by subjects prior to and during experimental encounters. Payne and Swanson (1971c and 1972c) have paired group-housed females in series of prolonged encounters (10 minutes/encounter). During encounters alone, each female experienced 60 min. of social contact with strange female conspecifics. In contrast, all of our females had been caged individually since 28-35 days of age. Further, encounters staged during our studies were brief (3 min.) and the total duration of social contact in an encounter series never exceeded 15 min. for adrex-ovariex females.

In experiments with naturally cycling females (Chapter II) we have seen that response frequencies and sequencing change over successive encounters. This change is most marked for behaviors indicative of relatively high levels of aggression (also see Lerwill & Makings, 1971) and probably stems largely from the formation of polar dominance-submissive relationships within pairs. Marked changes occurred within a series of brief encounters accounting for a total of 24 min. of social contact, of which only 18 occurred on nonestrous days. These observations suggest that social interactions occurring during encounters

described here may differ markedly from those in encounters summarized by Payne and Swanson (1971c and 1972c). Gross differences in the character of interactions may be accompanied by differences in the behavioral effects of physiological manipulations such as hormone treatment (also see Grelk et al., 1974). If hamsters are solitary, as suggested by the intense aggression displayed by both sexes, behaviors occurring relatively early during their social interactions may be more relevant to their natural behavior than are behaviors occurring much later than the time at which unconfined individuals probably would have terminated interaction.

Our use of adrenalectomized-ovariectomized females was designed to eliminate all endogenous sources of sex steroids. However, the maintenance of adrex-ovariex females depended on desoxycorticosterone replacement therapy. While this hormone (at doses of 10 mg/kg) does not affect isolation-induced aggression in mice (Kostowski et al., 1970), it has been reported to effectively mimic some effects of progesterone in hamsters (Czyba & Chiris, 1963; Isaacson, 1949; Tedford & Risley, 1950).

Thus, progestional effects of DOC might have maintained high levels of aggressiveness in our adrex-ovariex females and minimized effects due to additional P. This interpretation, however, requires that P exert virtually all-or-none facilitatory effects on aggression and that it operate with quite a low threshold. Assuming negligible retention of hormone beyond 10-14 days (consistent with changes in the health of pilot animals maintained with longer intervals between DOC injections), the average daily ration of DOC received by adrex-ovariex females in these experiments was 55-75 µg/day (roughly 3-5 mg/kg). As reported earlier, additional daily injections of 500 µg of P had no consistent effects upon the aggressiveness of our adrex-ovariex females. Further, since females receiving EB and P (plus DOC) performed lordosis much more readily than those receiving just EB and DOC, the interpretation above also requires very different thresholds for P effects on lordosis and aggression. Such a dichotomy is not supported by observations of Carter and colleagues (Carter & Schein, 1971; Carter, 1972 and 1973; Carter & Porges, 1974), in which

aggression and sexual receptivity seemed to vary concurrently under a variety of circumstances. These considerations impose rather rigorous demands upon the ability of DOC levels employed here to substitute for P and effect a full restoration of aggressive behavior in adrex-ovariex female hamsters. We conclude that, at least during initial encounters experienced by pairs of female hamsters, progesterone (like EB and TP) is not crucial for the exhibition of vigorous aggressive behavior.

EXPERIMENT 4: AGGRESSION IN HYPOPHYSECTOMIZED FEMALES

The appearance of vigorous aggression in adrenalectomized-ovariectomized females (Experiment 2) does not eliminate the possibility that pituitary hormones play important supportive roles in female hamster aggression (e.g., see Lazarus & Crook, 1973). In particular, it seemed possible that high levels of female hamster aggression require high levels of some pituitary hormone, and that aggressive behavior is inhibited whenever gonadotrophin release is inhibited by steroids (i.e., estradiol and progesterone). Observations of aggressive behavior in hypophysectomized females provided a direct test of this hypothesis.

Method

Subjects, Maintenance and Surgical Manipulation

Thirty-two adult (80-90 day old) random-bred female hamsters of the LVG:LAK strain were purchased from Lakeview Hamster Colony, Newfield, New Jersey, and shipped directly to Endocrine Laboratories, Madison, Wisconsin for hypophysectomy. All survived surgery and were in generally good health upon arrival at The Rockefeller University two days after hypophysectomy.

Hamsters were housed individually in stainless-steel cages measuring 25 X 18 X 18 cm. The routine maintenance of hypox females was identical to the treatment accorded adrex-ovariex females (see Experiment 2 of this chapter). This included biweekly injections of 0.75 mg of desoxy-corticosterone pivalate (DOC).

Hamsters were allowed at least 25 days for full recovery from surgery and the stresses of travel. Mortality during this period was negligible (1 female). Furthermore, individual females continued to gain weight at an average rate of 2.4 gm/week throughout the experimental period and most were quite indistinguishable from healthy intact females. Females were approximately 105-150 days old when tested during April-May.

Twenty-six females were assigned to 13 experimental pairs matched for body weight. Regular injections of physiological saline were begun 3 days before the first encounter experienced by each pair. Two injections of 0.10 cc were administered each day, one early and one late in the "dim" segment of the diurnal cycle. On the day of testing (the fourth day of injections), the first injection was administered during the first hour of dim lighting and the second 45 $\pm$ 10 minutes prior to testing during a period 3.5-6.5 hours after the initiation of dim lighting. The time of day at which each pair was tested remained constant ($\pm$ 30 min.) over subsequent tests.

Following an initial encounter at the end of a series of control NaCl injections, pairs of females were assigned randomly to one of the following hormone-treatment groups (all hormones were purchased from either Schwarz/Mann, Orangeburg, New York, or from Sigma Chemical Company, St. Louis, Missouri) adrenocorticotropic hormone (ACTH, 200 µg or 25 I.U./day); follicle-stimulating hormone (FSH, 300 µg or 0.3 Armour standard unit or 2.9 - 4.3 I.U./day, see Butt, 1967, pp. 105-106); luteinizing hormone (LH, 300 µg or 0.3 Armour standard unit or 450 I.U./day, see Butt, 1967, pp. 105-106); prolactin (PRO, 1 mg or approximately 20 I.U./day); or, saline control. These hormone regimes were selected so as to provide quantities of hormones comparable to those shown in other situations to exert behavioral effects (Mathewson, 1961; de Wied, 1966; Weiss, McEwen, Silva & Kalkut, 1969; Pfaff, 1969 and 1970b; Lazarus & Crook, 1973). The dosages cited above were divided between two daily injections administered as during the previous NaCl-control series. Hormones were dissolved in physiological saline, except for PRO, which required a special solvent (3 mg/ml Phenol and 50 mg/ml Glucose, in H_2O). Each injection was administered

subcutaneously and included a total volume of 0.1 ml. The first day of hormone treatment was four days after the initial (NaCl-control) encounter. This schedule was maintained throughout subsequent test series. Thus, encounters were staged at regular weekly intervals, each encounter-day preceded by 3 days of hormone-treatment and followed by a 3-day rest period.

Following their second encounters (the first under hormone replacement therapy) female-female pairs were randomly reassigned to the above hormone-treatment conditions. The following restrictions were enforced: (a) pairs treated with only NaCl during both of the first two encounter-series (2 pairs) continued to serve as injection controls, receiving only control NaCl treatment; (b) except for the 2 pairs given only NaCl, no hypox pair experienced the same hormone treatment during the second and third test series. Injections and aggression tests were scheduled as during the first two encounter series.

Finally, each of the 13 pairs of hypox females experienced a fourth encounter under the influence of control-NaCl injections. With the exception of previous experience in the experimental situation, this final series was an exact duplicate of the initial encounter series.

This schedule of testing resulted in each of 13 pairs of hypophysectomized female hamsters having experienced at least two aggressive encounters during control therapy with NaCl. Two pairs devoted their entire four encounters to the NaCl control condition. Thus, a total of 11 hypox pairs, each experiencing 2 encounters during replacement therapy with 2 different pituitary hormones, accounted for the following breakdown into four quasi-independent hormone-treatment groups: ACTH, 6 pairs; FSH, 4 pairs; LH, 6 pairs; and, PRO, 6 pairs.

Procedures and Analysis

Throughout this experiment, the staging of individual encounters, and the analysis of movies of these encounters were as described previously.

Verification of Surgical Procedures

Following the completion of their fourth encounter series, all 29 surviving hypox females, together with 2 intact females, were sacrificed and perfused. Their brains were removed carefully and the sella turcica and brain base were examined closely (with the aid of a dissecting microscope) for any evidence of incomplete hypophysectomy. No female retained fragments of pituitary tissue attached to the base of the brain. However, nearly all (90% of individuals) had one or more small fragments of tissue in or close to the sella turcica. All such fragments, together with the brain were carefully removed and preserved in formalin. Fragments were subsequently weighed and their weights compared with the weights of whole pituitaries from two intact females. Average weights of pituitary fragments recovered from hypox females were very small compared to average pituitary weights of intact females (mean = 3.3% of normal pituitary weight, standard deviation = 4.0%). Thus, it is clear that pituitary fragments surviving hypophysectomy were consistently very small. It seems highly unlikely that any substantial fraction of pituitary function could have been spared consistently in the experimental group of 26 hypox female hamsters. We conclude that operative procedures employed essentially resulted in functional hypophysectomies.

Statistics

Median latencies, and mean durations, contributed by different groups were compared using the Fisher exact probability test (Siegel, 1956).

Results

All 13 pairs of hypox females engaged in at least some intense fighting during control saline therapy. In fact, median latencies (Figures 21-22) and mean durations (Figures 23-24) of aggressive responses exhibited by these females are comparable to those typical of intact nonestrous females. Data based on direct comparisons of the first or fourth encounters experienced by members of these two groups are consistent with average patterns over entire series of encounters. If

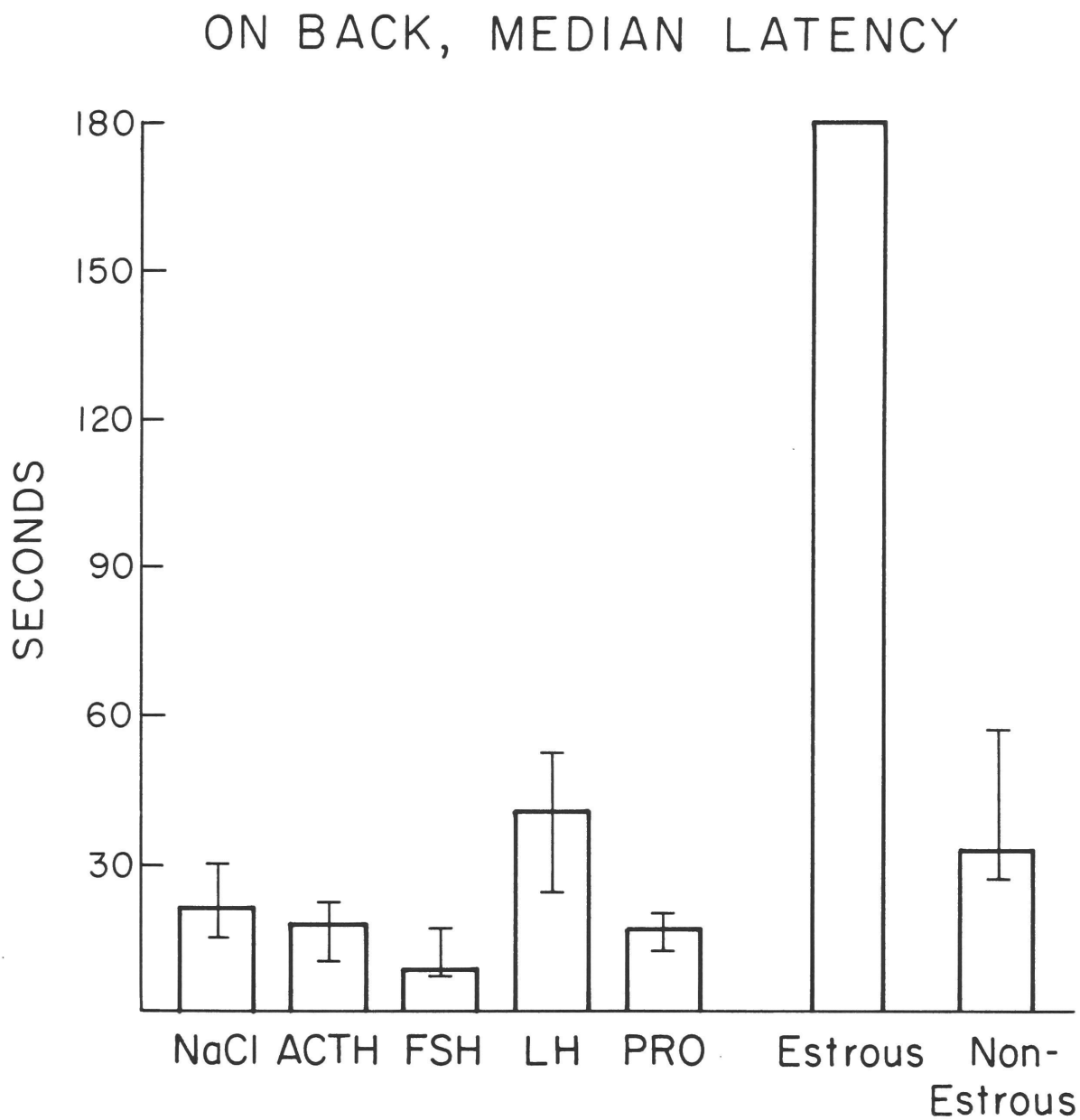


Figure 21. Effects of exogenous pituitary hormones on the median latency of ON BACK. Each of two pairs of hypophysectomized female hamsters experienced four 3-minute encounters during control NaCl treatment. Each of 11 pairs experienced two encounters during NaCl therapy, and two under different pituitary hormone regimes: adrenocorticotropic hormone (ACTH, 200 μg or 25 I.U./day); follicle-stimulating hormone (FSH, 300 μg/day); luteinizing hormone (LH, 300 μg/day); or, prolactin (PRO, 1 mg or 20 I.U./day). Median latencies of behavior on Estrous (Day 1) and Non-Estrous (Days 2-4) days of the normal estrous cycle are drawn from a previous experiment (see Figs. 8-10). Latencies corresponding to the first and third quartiles are specified, thus indicating the interquartile range of each distribution.

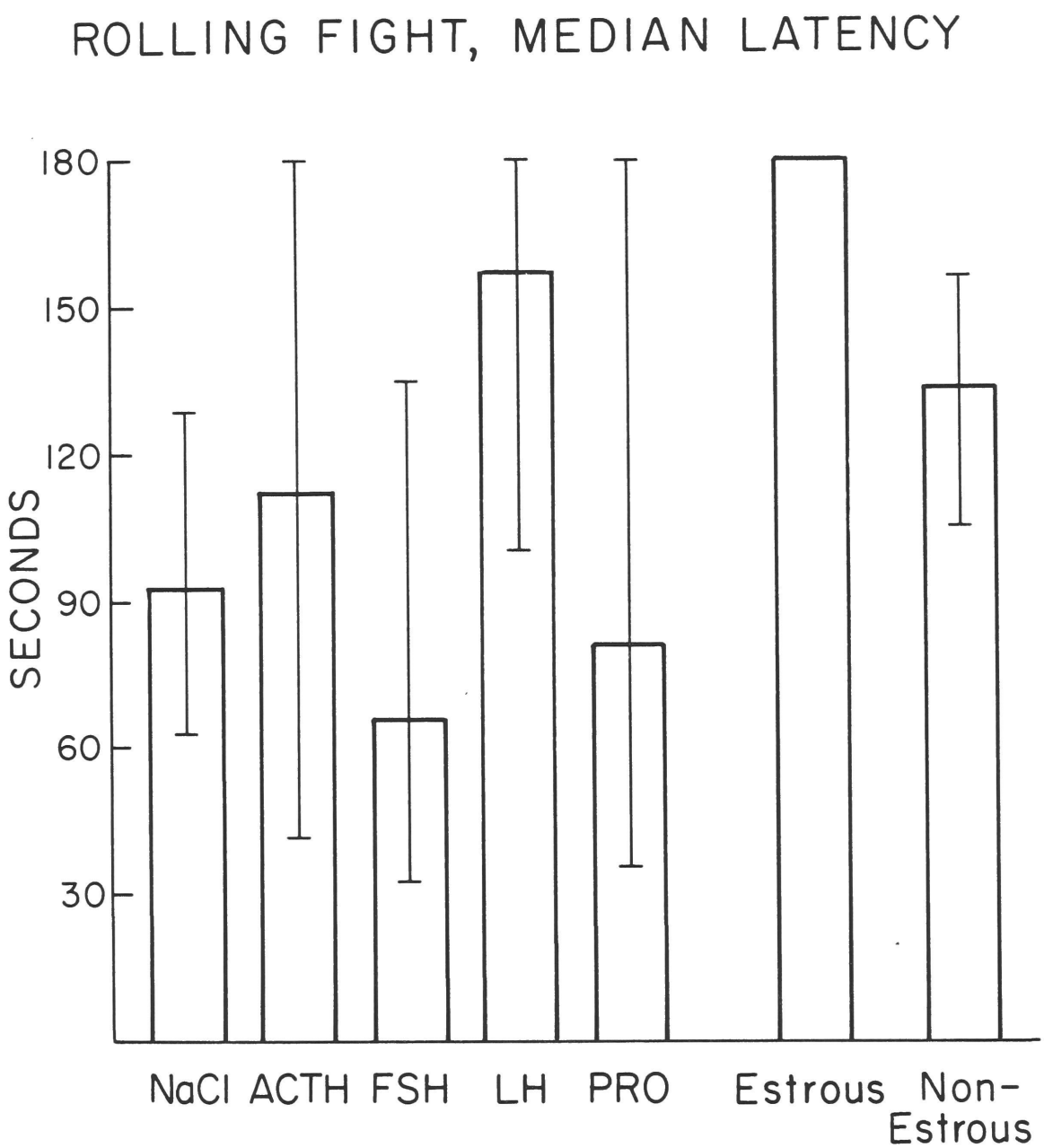


Figure 22. Effects of exogenous pituitary hormones on the median latency of ROLLING FIGHT. See legend of Figure 21 for details.

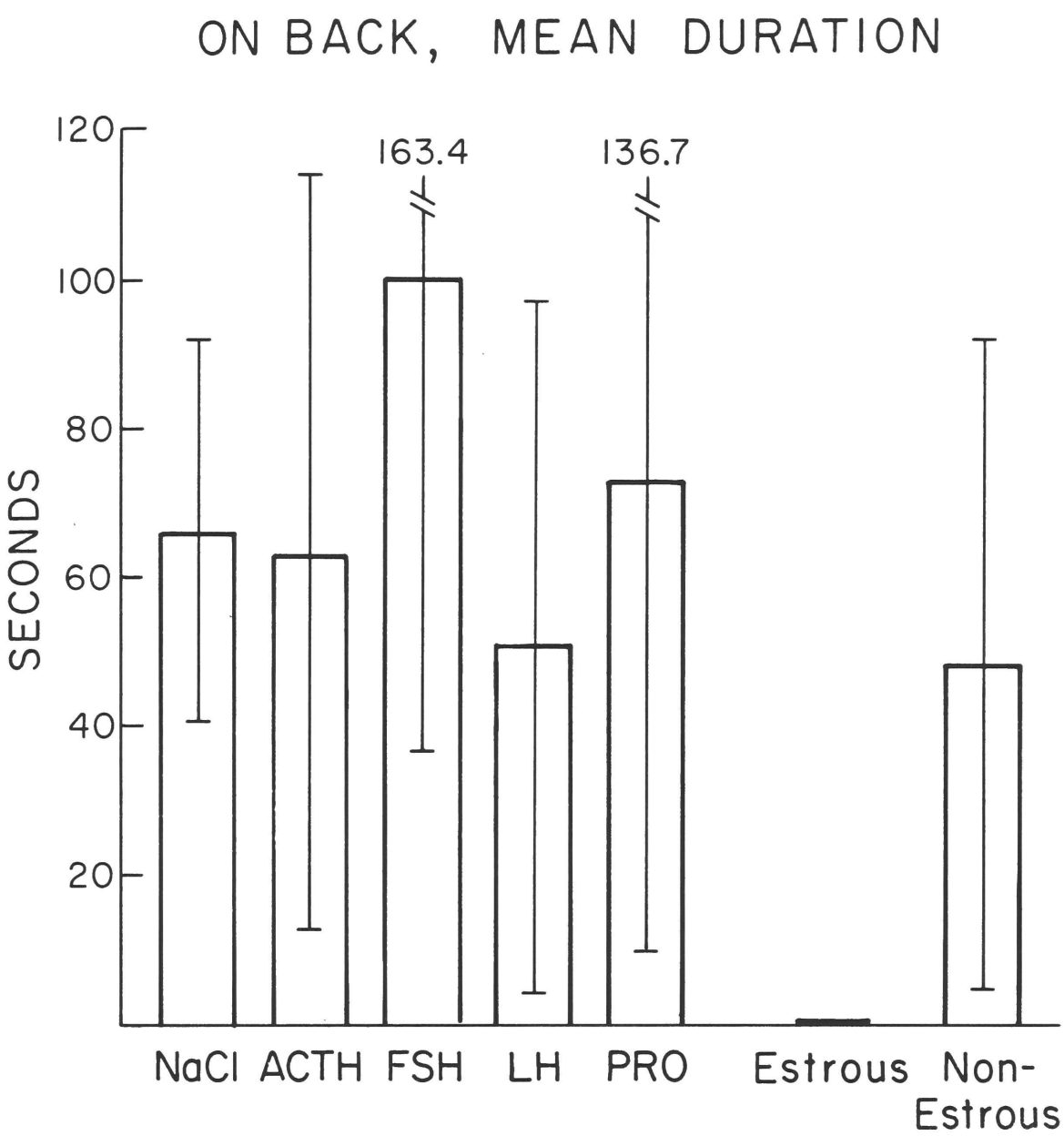


Figure 23. Effects of exogenous pituitary hormones on the mean duration of ON BACK. Each of two pairs of hypophysectomized female hamsters experienced four 3-minute encounters during control NaCl treatment. Each of 11 pairs experienced two encounters during NaCl therapy, and two under different pituitary hormone regimes: adrenocorticotropic hormone (ACTH, 200 μg or 25 I.U./day); follicle-stimulating hormone (FSH, 300 μg/day); luteinizing hormone (LH, 300 μg/day) or prolactin (PRO, 1 mg or 20 I.U./day). Mean durations (± 1 S.D.) under these regimes may be compared directly with those on Estrous (Day 1) and Non-Estrous (Days 2-4) days of the normal estrous cycle (also see Figs. 11-14).

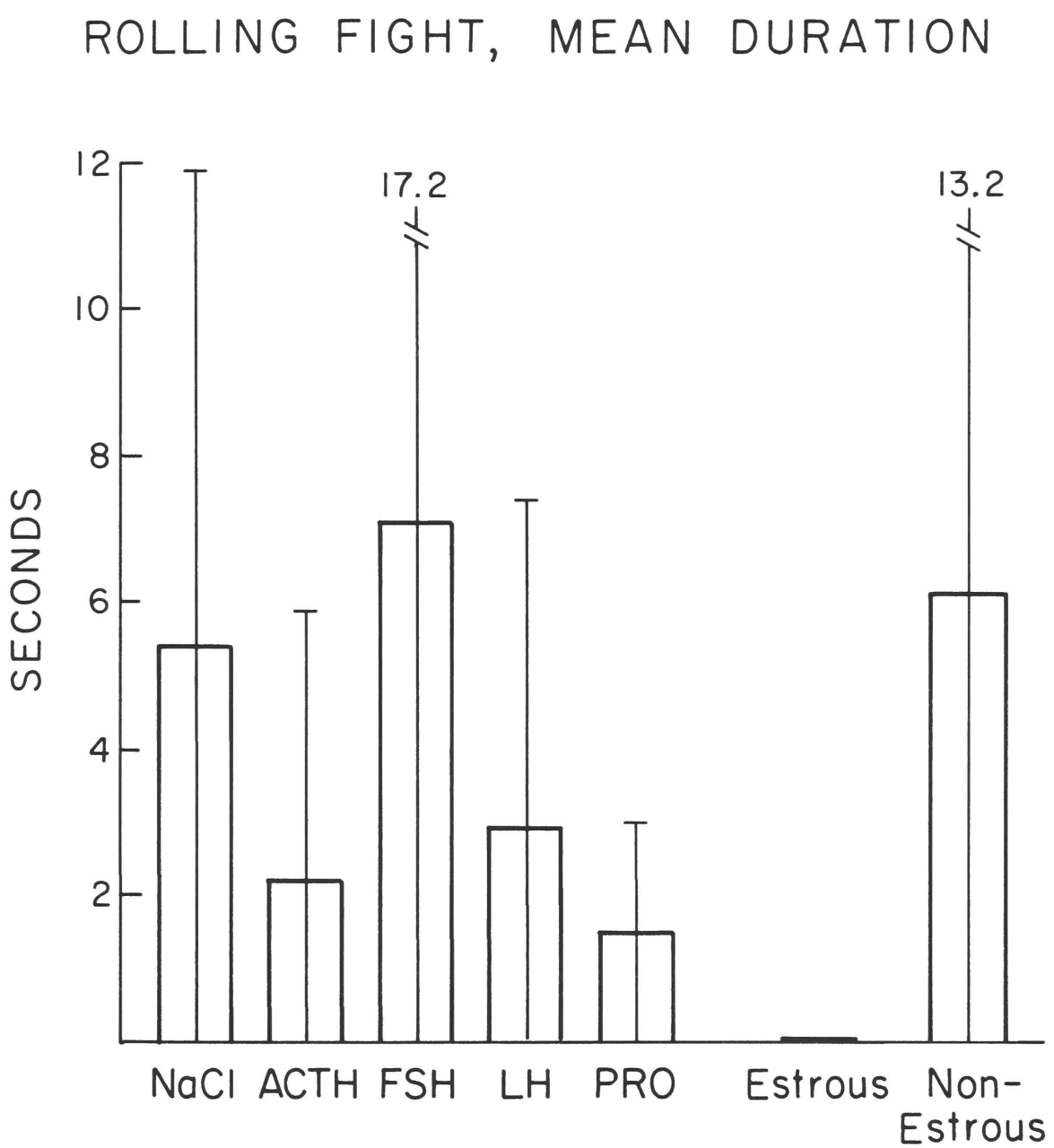


Figure 24. Effects of exogenous pituitary hormones on the mean duration of ROLLING FIGHT. See legend of Figure 23 for details.

anything, intact nonestrous females and hypox females treated with saline are even more similar in profiles of aggressive responses when comparable segments of their encounter series are considered. No comparison of average performance during the first hypox + NaCl encounter with the first intact nonestrous encounter yielded a statistically significant difference. Average levels of aggression exhibited during the fourth encounters experienced by each group were similar to the same degree.

The pituitary hormones ACTH, FSH, LH and PRO failed, at least with these dosages, injection schedules and testing conditions, to induce dramatic changes in female hamster aggressivity (Figures 21-24). Evaluation here may be hampered by the small size and limited statistical independence of hormone-treatment groups, since some trends seem to be relatively consistent across measures. For example, while not marked by any statistically significant differences, levels of aggression during replacement therapy with 300 μg/day of LH tended to be slightly, but consistently, lower than those characteristic either of intact nonestrous females or hypox females receiving only NaCl (Figures 21-24). The clarification of such trends will require future research devoting more attention to possible dosage-dependent effects and to possible interactions among different pituitary hormones in modulating aggression.

Discussion

With respect to our measures of hamster aggressive behavior, hypophysectomized females cannot be distinguished reliably from intact nonestrous females. Thus, it seems clear that the appearance of vigorous aggression in female hamsters does not require high levels of any pituitary hormone. The appearance of vigorous aggression among hypophysectomized females is consistent with the common occurrence of intense fighting during the nonestrous phase of the estrous cycle, when pituitary gonadotrophins are relatively low (e.g., Bast & Greenwald, 1974; Gay, Midgley & Niswender, 1970; and, Butcher, Collins & Fugo, 1974).

The exhibition of vigorous fighting by adrenalectomized-ovariectomized females (Experiment 3) and by hypophysectomized females suggests that the intense aggression characteristic of nonestrous female

hamsters represents a basal (hormone-free) state. However, increases or decreases in aggressiveness relative to this baseline could still depend upon pituitary hormones. For example, Wise (1974) has linked especially high levels of aggression exhibited by pregnant and lactating female hamsters to high levels of prolactin (also see Lazarus & Crook, 1973). Conversely, the inhibition of aggression in estrous female hamsters could be tied directly to high peripheral levels of pituitary FSH, LH or PRO. Peripheral levels of all three hormones are relatively low during nonestrous phases of the female cycle (when levels of aggression are relatively high), rising rapidly to sharp peaks during the preovulatory light period (Goldman & Porter, 1970; Turgeon & Greenwald, 1972; Bast & Greenwald, 1974; also see, Gay et al., 1970; and, Butcher et al., 1974).

The failure of direct manipulations of ACTH, FSH, LH or PRO levels to induce dramatic changes in the aggressiveness of hypophysectomized female hamsters suggests that no single one of these pituitary hormones is adequate to stimulate or suppress female aggression. Thus, any elevation in levels of female aggression associated with pregnancy or lactation may require factors other than (or in addition to) high prolactin titers. Conversely, the failure of exogenous ACTH to inhibit aggression, together with the similarity in levels of aggression exhibited by control hypox females and intact nonestrous females, suggest that ACTH does not inhibit aggression in the female hamster, as it seems to in male mice (Sigg, 1969; Brain et al., 1971; Leshner, 1972; and, Leshner et al., 1973). Finally, the failure of FSH, LH or PRO to markedly alter the aggressiveness of hypox females suggests that the suppression achieved by EB and P (Experiment 3) does not operate through the pituitary, but rather requires a direct interaction of these steroids with the brain.

GENERAL DISCUSSION

The mechanisms by which hormones affect aggressive behavior are unclear. Several probably contribute to the marked inhibition of aggression seen in receptive female hamsters:

A. Lordosis is a rigid and highly stereotyped posture. The performance of lordosis is inconsistent with the performance of any previously described hamster agonistic behavior. Thus, the lordosis posture constitutes a "competing response" which, by excluding aggressive acts, must contribute to the estrous female's low level of aggression (see also Svare & Leshner, 1973).

B. Hormones also may affect the level of aggression achieved during an interaction between two individuals by altering the stimulus properties of either contestant. Thus, hormones may raise or lower an individual's adequacy as an aggression-eliciting stimulus. For example, the simple immobility of an estrous female in lordosis may inhibit a viewer's propensity to attack (e.g., Alberts & Galef, 1973; Cairns & Scholz, 1973; Mettälä-Portin, 1966). The olfactory stimulus properties of an individual also are subject to hormonal modification. The facilitatory effects of androgens on an aggression-promoting pheromone in castrated male mice may be partially blocked by progesterone (Lee & Griffo, 1974). Similarly, the administration of P to gonadectomized male or female hamsters seems to decrease the level of aggressiveness either elicits from an intact male opponent (Payne & Swanson, 1971b and 1972b; also, Payne, 1974). Here, too, the relative dominance of two antagonists may be influenced by a hormonally induced change in the stimulus properties of one contestant.

C. Finally, hormones might also affect fighting by altering an individual's responsiveness to particular aggression-eliciting stimuli. It is this sort of change that might be labelled most universally as a change in an individual's "aggressiveness." For example, consider the similarities and differences between encounters involving two estrous females (both Day 1) and encounters pitting one estrous female (Day 1) against a nonestrous (Day 3) female. At any particular point during each encounter, one female typically will be in lordosis, while the other moves actively about the arena. Thus, the two types of encounters are similar in that (A) lordosis constitutes a "competing response" for only one female at a time, and, (B) the stimulus properties afforded by the female in lordosis are identical. The two encounters would seem to differ principally in the "aggressiveness" of the one active female

in each pair (i.e., her responsiveness to the stimulus complex presented by the female in lordosis). This difference can lead to very different levels of fighting in the two types of encounters: Several observations of single encounters between estrous and nonestrous females indicate that severe fighting may occur and that levels of aggressiveness are considerably higher than those typical of encounters between two estrous females (unpublished observations, also see Tiefer, 1970). Apparently, estrous female hamsters, like castrated male rats (Barfield et al., 1972) may fight effectively if sufficiently provoked.

These considerations indicate that each of the three suggested mechanisms through which hormonal influences on aggression might be effected probably operate in encounters between female hamsters. In fact, alternative mechanisms may be highly interdependent. In a highly organized chain of behavioral responses (Chapter II) any change in response topography, as a result of a change in level of aggressiveness or the induction of a competing response, may alter the opponent's stimulus complex sufficiently to alter her subsequent response and possibly break the chain. Our data suggest that the transition from an Upright posture (or some other preliminary posture) to an Attack might be such a hormone-sensitive link in the chain of hamster agonistic behaviors characteristic of female-female encounters.

CHAPTER IV. SIGNALLING SYSTEMS AND THE REGULATION OF FEMALE HAMSTER SOCIAL BEHAVIORS

The ability to describe encounters between anestrous female hamsters in terms of a chain of somewhat stereotyped response elements (Chapter II) suggests that the progress of a fight depends upon responses of each antagonist to signals from its opponent. Rapid communication regulating the progress of a fight might require visual (see Grant, Mackintosh & Lerwill, 1970; and Payne & Swanson, 1972d), auditory or somatosensory signals.

During more preliminary stages of a social interaction, specific communications may act as important determinants of relative success or dominance. For example, among red deer stags, (Lincoln et al., 1970 and 1972) the size and condition of antlers may be more important determinants of social status than androgen level (also see Bouissou, 1972). Similarly, while debeaked hens compete successfully with intact controls, the removal of a pullet's comb and wattles precipitates a decline in social rank (see Gottier, 1972).

Among nocturnal rodents, olfactory signals may affect the character and eventual outcomes of social interactions. As suggested in the preceding chapter, consideration of the female hamster as a source of olfactory cues seems relevant in interpreting certain effects of hormones on aggression. When paired with intact male opponents, the relative success of gonadectomized male or female hamsters may be enhanced by progesterone treatment (Payne & Swanson, 1971b and 1972c). In each case, however, the increased success of the progesterone-treated partner seems to be an indirect effect involving a decrease in the level of aggressiveness exhibited by the intact male opponent. These results are consistent with the notion of a progesterone-dependent olfactory cue capable of inhibiting the aggressiveness of male hamsters. Ample precedents for such signalling mechanisms (relevant particularly to sexual differences in the display and elicitation of aggressive behavior) are apparent in the work of Lee and colleagues (Lee & Brake, 1972; Lee & Griffo, 1972, 1973 and 1974), Mackintosh and Grant (1966) and Mugford and Nowell (1970a and b, 1971a and b, 1972). The latter authors have linked relatively high

levels of aggression displayed by isolated male mice toward androgenized females to the induction in those females of aggression-eliciting olfactory cues emanating from the preputial glands and other sources. In contrast, urine from intact female mice (Conner, 1972; and, Mugford & Nowell, 1970a) and a vaginal secretion of female hamsters (Johnston, 1972; and, Murphy, 1973) share the ability to suppress the aggressive behavior of male conspecifics.

The persistence of olfactory cues deposited during stereotyped scent-marking (e.g., Johnston, 1970a and 1974a-c) allows them to serve as distance signals, acting at locations remote from the present location of the communicator. In a variety of species, scent marking seems most closely related to aggression among and intolerance of conspecifics (Ralls, 1971). However, scent marks also may serve as sexual attractants. For example, female hamsters exhibit a stereotyped form of scent marking associated with the deposition of an odorous vaginal secretion (Johnston, 1970a). The frequency of "vaginal marking" and the viscosity of the vaginal discharge may be correlated with the estrous cycle (Johnston, 1970a; Orsini, 1961). Male hamsters show a distinct attraction to the odors of estrous female vaginal secretions (Gregory, Engel & Pfaff, 1974; Johnston, 1970a; Johnston, 1972; Murphy, 1973). Under ideal conditions, this attraction is at least as pronounced as that shown by sexually experienced male rats for estrous female rat urine odor (Pfaff & Pfaffmann, 1969). Even sexually inexperienced intact male hamsters can show a significant preference for the estrous odor (Gregory et al., 1974; Johnston, 1970a). Moreover, the degree of preference exhibited by male hamsters for the estrous odor is reduced following castration and can be reestablished with testosterone replacement therapy (Gregory et al., 1974). Such an odor might not only attract male hamsters as mates, but also inform potential opponents of both sexes about the state of the female's estrous cycle, linked both to sexual receptivity and aggressivity. Potential mates, in particular, might be encouraged to concentrate sexual advances on estrous days, thus avoiding contact with aggressive anestrous females.

More socially tolerant, or gregarious species, might have little need for distance communications related to sexual attraction. However, nonestrous female hamsters are quite indiscriminate as to the objects of their aggression. In fact, the aggressive behavior exhibited by nonestrous females in male-female pairings is similar in form and intensity to that seen during female-female interactions (e.g., Chapter III; Payne & Swanson, 1970). The intense aggression which dominates intraspecific social interactions among hamsters suggests that free-ranging individuals of this species lead a largely solitary existence. The maintenance of such a social organization might depend upon communications which announce the overall state of receptivity and aggressiveness of a female without requiring close physical contact with her. Such noncontact signals might function most economically by attracting potential mates on a female's estrous day. In this regard, we have found that hamsters of both sexes emit high frequency (ultrasonic) calls which could function primarily as sexual attractants (Chapters V-VIII).

CHAPTER V. PHYSICAL CHARACTERISTICS OF HAMSTER ULTRASOUNDS

Virtually all species of small rodents examined produce, and apparently hear, sounds with frequencies well above the range of human hearing (e.g., Schleidt, 1952; Anderson, 1954; Zippelius & Schleidt, 1956; Ralls, 1967; Noirot, 1970 and 1972; Barfield & Geyer, 1972; Sales, 1972a and b; Brooks & Banks, 1973; and, Brown, 1973a and b). For example, infant Peromyscus maniculatus emit partly audible (8-29 kHz) and purely ultrasonic (60-140 kHz) signals in response to cold stress and tactile stimulation, respectively (Smith, 1972). Noirot (1972) has suggested that distinct types of ultrasonic calls emitted by rodent pups might correspond to different maternal responses. In particular, calls emitted during cold stress might elicit approach and retrieval, while those stemming from tactile stimulation might provoke withdrawal, with cessation of rough handling or aggression.

Adult male house mice (Mus musculus) emit high-amplitude ultrasounds (at approximately 70 kHz) during heterosexual encounters (Sales, 1972a; Whitney, Coble, Stockton & Tilson, 1973; Whitney, Alpern, Dizinno & Horowitz, 1974). Their appearance at about the age of male sexual maturity, and their dependence on female olfactory cues, suggests an involvement at an early stage of male courtship and mating behavior (Whitney et al., 1973 and 1974). In contrast, the appearance of a 22-30 kHz male rat call in conjunction with post-ejaculatory abstinence during heterosexual interactions and its emission by defeated males in aggressive encounters support interpretations of this call as a "desist-contact" signal (Barfield & Geyer, 1972; Sales, 1972b).

During preliminary observations, we found that approximately 20% of adult female hamsters in our colony emitted distinctive audible vocalizations when sexually receptive. These audible vocalizations were quite variable in their physical characteristics (as judged informally by a human listener). While sharp barks occasionally were emitted, forms resembling low hisses or "huffs" (see Rowell, 1960) were more typical. This latter type of sound often is associated with the presence of high-frequency components. The physical characteristics of hamster audible calls, then, suggested that high-frequency signals might be involved in

social communication among hamsters. In particular, we hypothesized that these distinctive vocalizations might function to attract males to a sexually receptive female, perhaps in part by signalling relatively low levels of female aggressiveness.

Sales (1972a and b; also see Sewell, 1970) also has described high-frequency sounds (20-50 kHz and 50-200 msec) emitted by hamsters during homosexual and heterosexual social interactions. "Pure" ultrasounds (signals with all components exceeding 20 kHz) have been attributed only to estrous female hamsters. In contrast, males have been reported to emit only wide-band signals (possibly screeches and screams) during male-male and male-female aggressive interactions. In this respect, hamsters seem unique among the rodent species surveyed. High-frequency vocalizations by adult rodents most frequently are the prerogative of males, and they typically accompany both sexual and aggressive interactions. The frequent emission of such sounds by estrous female hamsters has been linked to high levels of intra-specific aggression and a solitary mode of existence: "These animals are normally solitary and are generally very aggressive to other individuals. The ultrasonic signals may therefore be important in advertising an oestrous female's temporary lack of aggression and her willingness to mate" (Sales, 1972a, p. 162).

We have found that hamsters of both sexes can emit pure ultrasounds. Further, detailed consideration of (i) the physical properties of hamster vocalizations, (ii) the stimuli capable of provoking high rates of calling by male and female hamsters (Chapter VI), and (iii) the responses of male and female hamsters to natural and artificial vocalizations (Chapter VII), all support our hypothesis and the conclusions of Sales (1972a and b), suggesting strongly that these vocalizations could function as sexual attractants. The first step in this analysis was the compilation of a more complete and accurate description of the physical characteristics of the high-frequency calls themselves.

GENERAL METHOD, EXPERIMENTS 5-6

Subjects and Maintenance

Random-bred male and female hamsters, of the LVG:LAK strain, were purchased from Lakeview Hamster Colony, Newfield, New Jersey. Individuals were 28-63 days old when acquired and each was housed separately in a metal cage of dimensions 25 x 18 x 18 cm or 30 x 30 x 18 cm. Purina rat chow and tap water were supplied ad libitum. Cabbage, sunflower seeds and Purina guinea pig chow were supplied at weekly intervals.

Lighting in the colony rooms varied according to a reversed 12-hour bright:12-hour dim cycle. Temperature fluctuations were attenuated, ranging normally between 20 and 25 degrees centigrade, with extremes of 12 and 32 degrees over a period exceeding two years.

Tests for the emission of high-frequency sounds were conducted in 30 x 30 x 18 cm metal cages or in 48 x 27 x 20 cm polycarbonate cages. Participants ranged between 135 and 385 days of age. All were intact, and all females maintained regular 4-day estrous cycles throughout testing (see Orsini, 1961).

EXPERIMENT 5: INTENSITY MEASUREMENTS

Hamsters exhibit several forms of stereotyped scent-marking (Johnston, 1970). Persistent olfactory cues deposited during these behaviors might provide an effective means of communication over relatively great distances (e.g., Marler & Hamilton, 1966; also see Johnston, 1970a, and Chapter VIII). Less durable, but more easily localized, acoustic signals might serve most effectively as "non-contact" communications, operating over moderate distances to facilitate the achievement (or maintenance) of close social contact. A rough estimate of the effective range of hamster acoustic signals may be achieved through measurements of the sound pressure levels of these calls.

Method

Instrumentation

A wide-band (15-200 kHz) high-frequency receiver designed by the Lincoln laboratory of Massachusetts Institute of Technology (McCue & Bertolini, 1964) served as a transducer during intensity measurements and tape-recordings of hamster sounds. The intensities of high-frequency sounds were determined with reference to a calibrated condenser microphone manufactured by Bruel and Kjaer of Naerum, Copenhagen, Denmark (a B & K 1/4-inch condenser microphone, type 4135, used in conjunction with a B & K cathode follower, type 2615, and a B & K microphone amplifier, type 2604). Sound intensities were expressed with reference to a conventional scale of sound pressure levels in which 0 dB SPL corresponds to 2×10^{-4} dyne/cm^2 (or 2×10^{-5} Newton/m^2, or 2×10^{-4} μbar RMS: roughly equal to the minimum sound level detectable by a human observer under ideal conditions).

Natural hamster sounds were too brief to allow direct intensity determinations using the calibrated Bruel and Kjaer system. Thus, during intensity measurements the output from the McCue and Bertolini (M & B) ultrasonic receiver was monitored visually on a Tektronix, Inc. storage oscilloscope (type 564B). The maximum peak-to-peak voltages associated with individual calls were noted, and the average maximum peak-to-peak voltage was calculated for each subject. Subsequent to these initial measurements, and with oscilloscope and M & B gain settings fixed, a constant tone of 35 kHz (apparatus detailed below) was generated which provoked an M & B output identical in peak-to-peak voltage to the average value observed for a sample of natural calls. The absolute intensity (rms sound pressure level re 2×10^{-4} dyne/cm^2) of this constant tone could then be measured easily with reference to the calibrated Bruel and Kjaer system. The resulting measurement clearly is a rough estimate of the mean rms intensity of the sample of natural calls. An accurate matching of the maximum peak-to-peak excursion of a complex wave (such as a natural sound) with the peak-to-peak excursion of a regular 35 kHz sinusoid does not guarantee that the rms sound pressure levels of the two coincide exactly. Aside from

this factor, however, the choice of a 35 kHz tone, rather than a tone of any other frequency, should not have affected our measurements, since the relationship between the peak and rms pressures of a sine wave are independent of frequency.

Constant tones of 35 kHz were generated by an oscillator (Hewlett-Packard 200 CD), a 34-36 kHz band-pass filter (Allison Laboratories 2-CR), voltage amplifiers (General Radio 1206 and Krohn-Hite DCA-50), and an electrostatic loudspeaker (Kuhl, Schodder & Schroeder, 1954). The accuracy of frequency production by this system was checked with a Tektronix oscilloscope (type 502a).

Procedures

Two estrous female hamsters participated in measurements leading to an estimate of the average intensity of hamster ultrasounds. Immediately before testing, each female was "primed" to emit high-frequency calls at a high rate by a brief (30-60 second) exposure to an intact male (see Chapter VI). During initial measurements, the microphone of a M & B ultrasonic receiver was held as close as possible (5-15 cm) to the muzzle of each female in turn. The M & B output was monitored visually as noted above. A total of 36 calls were sampled in this manner, and observations of each call included an estimate of the female's exact location relative to the probe at the instant the call was recorded.

A second set of intensity measurements immediately followed the first, and were conducted on the same two estrous females. Here, the M & B probe was fixed at a distance of 35-50 cm from the test female (depending on her location within the test cage). Again, high rates of calling were provoked by brief pre-exposure to an adult male. Measurements of call intensity were conducted as detailed above for 20-21 individual calls by each female.

Results summarized below clearly represent quite rough estimates of the absolute intensities of hamster ultrasounds. Certainly, these estimates are based on a very small sample (of calls and females) drawn from a particular category of hamster (estrous females) observed under very special stimulus conditions. On the other hand, the particular

females discussed here did not differ from other estrous females in other aspects of their reproductive behaviors, including the time-courses and patterns of frequency modulation characteristic of their high-frequency vocalizations. Furthermore, even such rough estimates of call intensity may place useful limits on the effective ranges of hamster calls, thus contributing to their functional interpretation.

Results

The mean intensity of female hamster high-frequency sounds, observed at distances of 5-15 cm from an individual, was 54 dB SPL (range = 48-62 dB SPL). Comparable measurements at 35-50 cm from each of the two females yielded a mean sound intensity of 52 dB SPL (range = 42-61 dB SPL).

Discussion

During our observations, female hamsters have emitted high-frequency sounds at intensities of approximately 53 dB SPL at distances of 5-50 cm (overall intensity range = 42-62 dB SPL). Variability in call intensity probably stems in part from variable call output by individual hamsters. However, it also may reflect uncontrolled variations in (a) subject-to-microphone distance, and (b) the orientation of hamsters relative to the microphone at the time of call production. Both problems were exacerbated by the tendency of females to call while engaging in rapid and very abrupt movements of the head and body. For instance, many calls occurred during transitions between short darting runs and adoption of upright investigatory postures. Factor (b) may be especially important in view of known directional differences in microphone sensitivity and probable directional differences in emitted sound intensity. Sound reflection by the walls of the closed cages (48 x 27 x 20 cm polycarbonate cages open only at the top) used as test chambers also may have contributed to variability in apparent call intensity. In addition, such reflections could have accounted for the relatively small decrease in average sound intensity (from 54 to 52 dB SPL) with a change in subject-to-microphone distance from roughly 10 to 40 cm (an intensity change of 16 dB would have been expected in open air on the basis of the inverse square law).

Measurements of absolute sound pressure levels characteristic of rodent ultrasounds are rare. This, in part, probably reflects the commonality of problems such as those outlined above. Nevertheless, available evidence suggests that the intensities of sound emitted by hamsters are low compared with adults of other species. For example, adult male rats emit 22-23 kHz pulses with maximal intensities at least as high as 80 dB SPL, measured 5-10 cm from the individual's head (Barfield & Geyer, 1972). Similarly, adult male mice emit 70 kHz sounds with intensities greater than 60 dB SPL measured 25 cm or more from the individual (Whitney et al., 1973 and 1974).

While generally less intense than the high-frequency sounds emitted by other rodents, hamster calls still could serve as non-contact communications over moderate distances. Limited knowledge of hamster ecology and acoustic sensitivity obviates a precise estimate of the effective range of these vocalizations. However, under ideal conditions, tones of 30-40 kHz (see Experiment 2) are subject to atmospheric absorption of 0.57 - 0.83 dB SPL/meter (Griffin, 1971; Evans & Bass, 1972). To calculate the net rate of attenuation in open air, this factor must be added to the more familiar spreading loss suffered by spherical waves (described by the inverse square law). Considering both sources of attenuation, a hamster call emitted at 60 dB SPL and 30-40 kHz would be attenuated to 0-10 dB SPL over a distance of 11-23 meters. Since individuals of at least some rodent species exhibit inferior collicular thresholds approaching 10 dB SPL to high-frequency tone-pulses (Brown, 1973b), acoustic thresholds of 0-10 dB SPL seem reasonable for intact hamsters tested under ideal conditions. In turn, we feel that 11-23 meters represents a reasonable estimate of the effective range of hamster calls under ideal conditions for open-air sound transmission. In more natural settings, the information value of high-frequency sounds emitted at ground level might be attenuated more rapidly as a result of sound scattering by obstacles, while ultrasounds emitted within a burrow system might be transmitted much more efficiently than in open air because of their relative freedom from spreading loss. In any case, our calculations suggest that female hamster "ultrasounds" are sufficiently intense to serve effectively

as noncontact signals advertising the location of a nearby female. For a species with alternative modes of long-distance communication (e.g., Johnston, 1970a), the limited range of high-frequency acoustic signals might aid in restricting elicited responses to conspecifics, rather than predators.

Finally, basic questions remain unanswered regarding the exact mechanism of production of sounds such as those described above (Sewell, 1970). Hamster ultrasounds occasionally are emitted in the absence of any detectable locomotion on the part of the source. They are most intense when the individual's head is close to and oriented toward the recording probe. Ultrasounds may be emitted during olfactory exploration of the environment (sniffing), or during self-grooming, as well as in response to particular stimuli presented at specific points in the estrous cycle. Sounds which contain components resembling some segments of hamster calls and which elicit similar responses as are provoked by natural calls, may be produced by humans during rapid, pulsed exhalation through tightly pursed lips. All of these observations indicate that the vocal and/or nasal passages are probably involved integrally in the production of high-frequency sounds by hamsters. They suggest further that such sounds may require abrupt, forceful exhalations (or inhalations) of air, possibly with vocal or nasal tracts acting primarily as simple resonance filters.

EXPERIMENT 6: FREQUENCY, DURATION AND WAVEFORM DETERMINATIONS

Patterns of amplitude and frequency modulation contribute to the effective ranges of natural sounds and can distinguish signals with different messages. Sound spectrographic analyses of vocalizations by female and male hamsters represented the first step in the investigation of these patterns.

Method

Instrumentation

Tape recordings of female hamsters or male-female pairs were made for the analysis of frequency and amplitude distributions within individual hamster calls. As in the case of measurements of absolute

sound intensities (Experiment 5), a wide-band M & B ultrasonic receiver (McCue & Bertolini, 1964) served as a transducer of high-frequency sounds. With the M & B microphone held approximately 5-15 cm from the sound source (a female hamster or male-female pair), the receiver's output was filtered by a Krohn-Hite filter (3500) operating with a pass-band of 15-100 kHz. This filtered signal then was tape-recorded at 60 inches-per-second (ips) on a Precision Instruments tape recorder (PS 202). The accuracy of this entire recording system was checked for sinusoidal input signals of 15-70 kHz. The tape recorder output was monitored on an oscilloscope, and the accuracy of frequency reproduction was confirmed for tape speeds of 60 and 7.5 ips (used for the recording and reproduction of high-frequency sounds, respectively). However, the fidelity with which fine details of the input waveform were reproduced depended on the amplitude of the input signal. For input signals of 35 kHz (a frequency commonly emphasized in natural hamster calls, see Results) and approximately 2-6 volts, amplitudes such as those delivered to the tape recorder during most observations of natural hamster sounds, the frequency of the input signal was preserved accurately, but minor alterations in waveform were evident when compared to the original sinusoid.

For the description and analysis of prominent physical characteristics of hamster ultrasound, tapes were played back at a speed of 7.5 ips, reducing the frequency of recorded signals by a factor of 8 and bringing all natural sounds of at least 15-70 kHz into the human audible range. Hamster calls with adequate signal-to-noise ratios were selected on the basis of acoustic and visual inspection. The frequency distributions of these calls were displayed in sound spectrograms (sonagrams) made on a Kay Sona-Graph (model 7029 A) set for a frequency range of 15-64 kHz and resolution of $\pm$ 1.2 kHz (wide-band). The locations of calls selected for spectrographic analysis were marked on the tapes with white ink. Many subsequently were displayed on a Tektronix oscilloscope (RM565) and photographed with continuously moving film (Grass CH-K camera). Photographs of the oscilloscope traces of some calls later were enlarged and their time scales adjusted to coincide with sonagram

scales. The availability of corresponding sonagrams and oscilloscope traces allowed the direct comparison of patterns of frequency and amplitude modulation exhibited by many hamster calls representative of a variety of situations.

Procedures

Spectrographic analyses of hamster ultrasounds were performed on tape-recordings of female hamsters or male-female pairs. Tests were conducted in a quiet, dimly-lit room. A typical test included: (a) 5 minutes of acclimatization to the test room; (b) a 30-second period during which a female of known estrous state (Orsini, 1961) was observed prior to the introduction of a male; (c) a 30-second period during which an intact adult male was permitted to interact freely with the test female. If lordosis occurred, its latency was noted. During subsequent analysis, this allowed observed calls to be assigned to periods (i) before, or (ii) after, the adoption of lordosis by the female member of the pair. Estrous (Day 1) females often adopted lordosis within 10-15 seconds of the male's introduction, and always maintained this rigid posture throughout the remainder of the 30-second period. Thus, test segment (ii) corresponds to a period during which the female was continuously in lordosis, and the male was the only active member of the pair. Later results (Chapter VI) indicated that estrous females do not vocalize while in the rigid lordosis posture, thus permitting us to attribute calls emitted during this period to the male; (d) following the removal of the stimulus male, test females were left undisturbed for a sufficient time to emerge from lordosis. At the instant active locomotion was resumed, a 1-minute observation period was begun; (e) a second minute of observation followed immediately. However, here, the observer emitted one imitation hamster call (a rapid pulsed exhalation through tightly pursed lips) every 15-seconds, for a total of four during this final test segment.

During each observation period (a-e above), the microphone of a M & B ultrasonic receiver was held as close as possible (5-15 cm) to the female or male-female pair. A high-speed tape-recording of the M & B output was made as indicated above.

Subjects and Analysis

Ten female and 3 male hamsters participated in a total of 25 tape-recorded tests (19 on estrous days, 6 distributed over the 3 nonestrous days of the female sexual cycle). Following an initial review of the resulting tapes, a total of 10 tests (6 on estrous days, 4 distributed over the 3 nonestrous days of the female cycle), involving 6 different females and 3 males, were selected for more detailed analysis.

Sound spectrograms were made of 75 individual hamster vocalizations. A total of 46 calls were emitted by 6 different estrous females (4 immediately before male-female contact and 42 following the male's removal). The highest rate of calling, and the largest number of calls analyzed (35), were associated with the post-male test segment during which the experimenter produced imitation hamster calls at regular intervals. Natural and imitation calls were easily distinguished, even on the frequent occasions when imitation calls were followed closely by one or more natural female call. Since female vocalizations in response to imitation calls did not differ consistently from those emitted earlier in the post-male period, these were not distinguished in subsequent analyses.

Calls produced by nonestrous females accounted for 18 of those subjected to spectrographic analysis. These were contributed by 4 different females, each of which also was responsible for some estrous-day calls. All of these nonestrous calls occurred during the test segment following brief exposure to a male, and most probably were emitted in response to imitation calls produced by the experimenter.

Only 3 different male hamsters participated in tests leading to a physical description of hamster high-frequency vocalizations. Further, all 11 male calls subjected to spectrographic analysis were produced by a single adult male, during 4 successive tests with 3 estrous females. Clearly, the present observations have focused on calls emitted by females, especially estrous females.

Quantitative Measures

Sonagrams of hamster vocalizations were examined with special attention to the following parameters: (a) the lowest, or minimum, frequency occurring during a call; (b) the maximum frequency achieved during a call; (c) the "dominant" frequency, a rough estimate of the average frequency of that call component containing the most sound energy (the darkest component on a typical sound spectrogram); (d) the total duration of the call; (e) the duration of the longest "gap" (period of near or complete silence) within each call (these gaps always were much shorter than the silent intervals separating adjacent calls); and, (f) each coherent segment of a call was examined closely and the point characterized by the most rapid change in frequency (the greatest slope on the sonagram) was noted. For each call, we then determined the largest change in frequency during any 10 msec interval.

Statistics

High-frequency vocalizations by male and female hamsters have been examined for consistent sexual and situational differences using Chi-square and t-tests for dependent samples (Siegel, 1956; Edwards, 1966). All statements regarding the statistical significance or chance probabilities of observed differences have been based on two-tailed tests of significance.

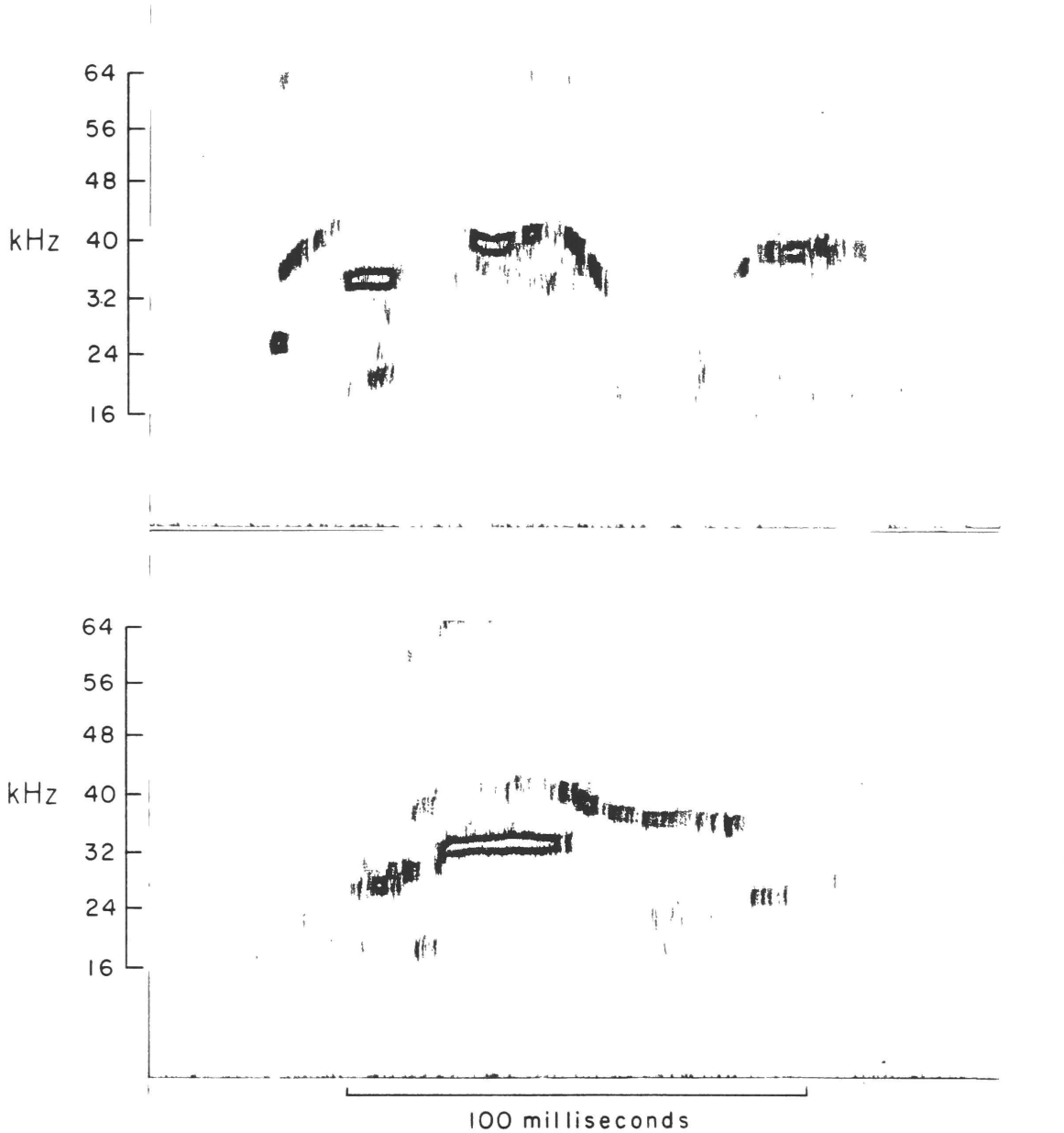
Results

Some prominent physical characteristics of hamster high-frequency sounds are summarized in Table V, and are readily apparent in representative sonagrams and oscilloscope traces included in Figures 25-30. For example, it is clear that hamster ultrasounds are quite variable in frequency. While calls from a variety of sources emphasized frequencies within the narrow range of 30-40 kHz (Table V), most had bandwidths of at least 25 kHz. Thus, components of these calls extended over a frequency range of 17-64 kHz. Even this probably underestimates the full range of frequencies utilized by hamsters. Informal

Table V: Physical Characteristics of Female and Male Hamster Ultrasounds, Mean (SEM*)

ɔurce	Frequency (kHz)			Duration (msec)		Most Rapid Change in Frequency (kHz/10 msec)
	Minimum	Maximum	Dominant	Total	Longest Gap	
female, pre-male emales, .11s = 4)	25.5 (1.7)	59.8 (3.2)	42.0 (12.4)	99.0 (33.8)	41.5 (13.6)	14.3 (8.4)
female, post-male emales, .11s = 37)	23.8 (1.0)	46.7 (1.7)	33.9 (1.8)	99.8 (13.5)	11.3 (4.8)	6.7 (0.5)
us female, e (n = 4 females, .11s = 18)	24.0 (1.5)	51.8 (3.9)	35.9 (2.0)	79.7 (23.2)	11.3 (8.4)	6.7 (0.8)
.le calls emales, .11s = 59)	23.6 (0.7)	49.4 (2.2)	35.4 (1.7)	97.0 (11.4)	11.0 (4.0)	6.8 (0.4)
male present .ale, .11s = 11)	25.3 (1.5)	53.6 (1.4)	36.9 (2.1)	154.0 (12.5)	1.0 (0.5)	3.4 (0.5)

*Standard errors of female calls describe variability among individual females; however, standard errors of male calls summarize variability among different calls by a single individual.



Figures 25-28. Representative sonagrams of high-frequency sounds emitted by estrous female hamsters following brief exposures to males. In Figures 26-28, sonagrams are accompanied by oscilloscope traces of the same female calls. Oscilloscope traces are mounted directly above, and approximately in register with, the corresponding sonagrams. While sound frequency is depicted on the vertical axis for each sonagram, vertical deflections in oscilloscope traces indicate relative sound pressures or intensities. For both representations of hamster ultrasounds, time is depicted along the horizontal axis. All calls were recorded at a speed of 60 inches per second and reproduced at a speed of 7.5 inches per second. The analyzing filter bandwidth employed in making sonagrams is equivalent to approximately 3.4 kHz. The total range of frequencies sampled in these sonagrams is 16-64 kHz.

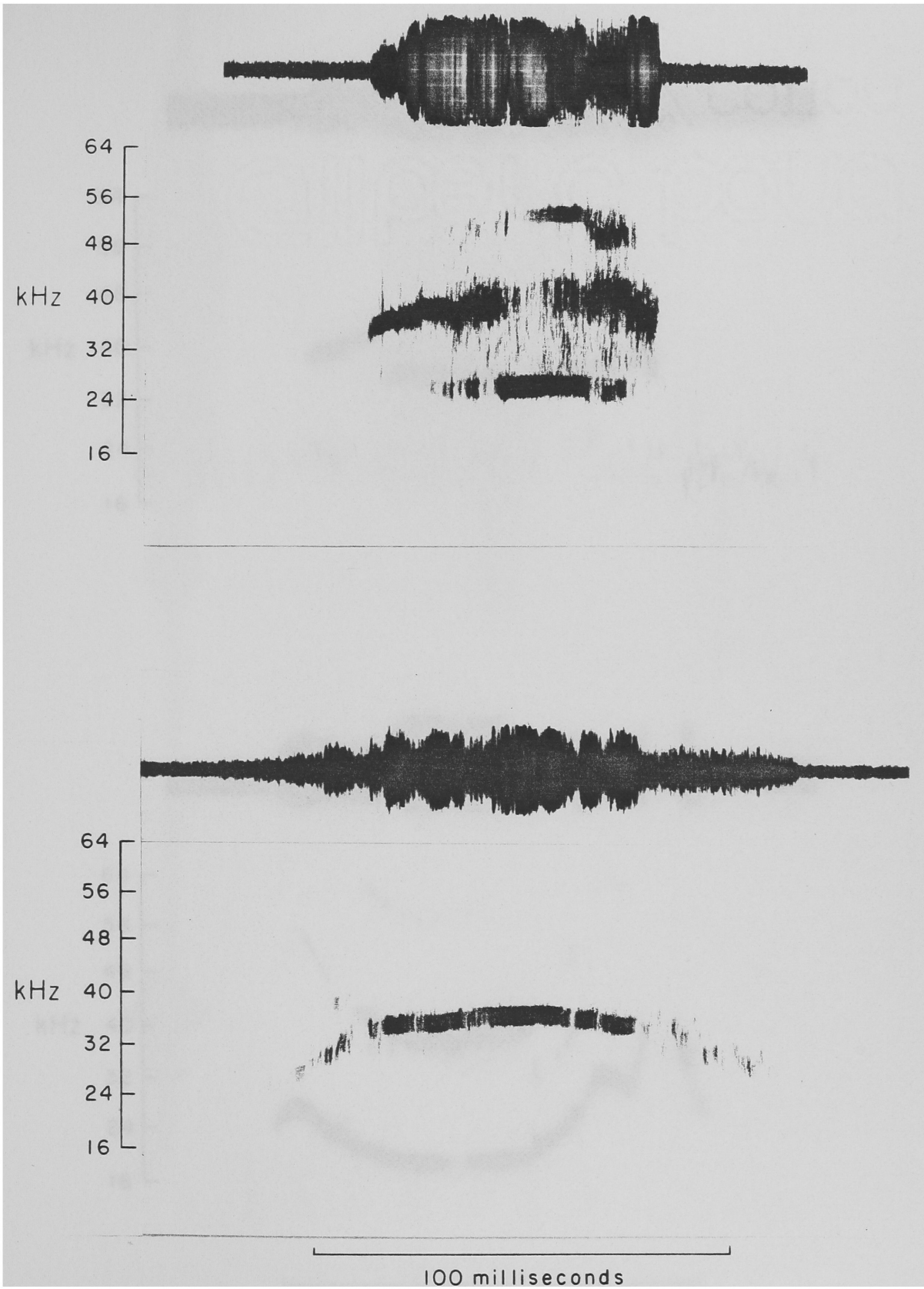


Figure 26. Representative sonagrams of high-frequency sounds emitted by estrous female hamsters following brief exposures to males. See legend of Figure 25 for explanation.

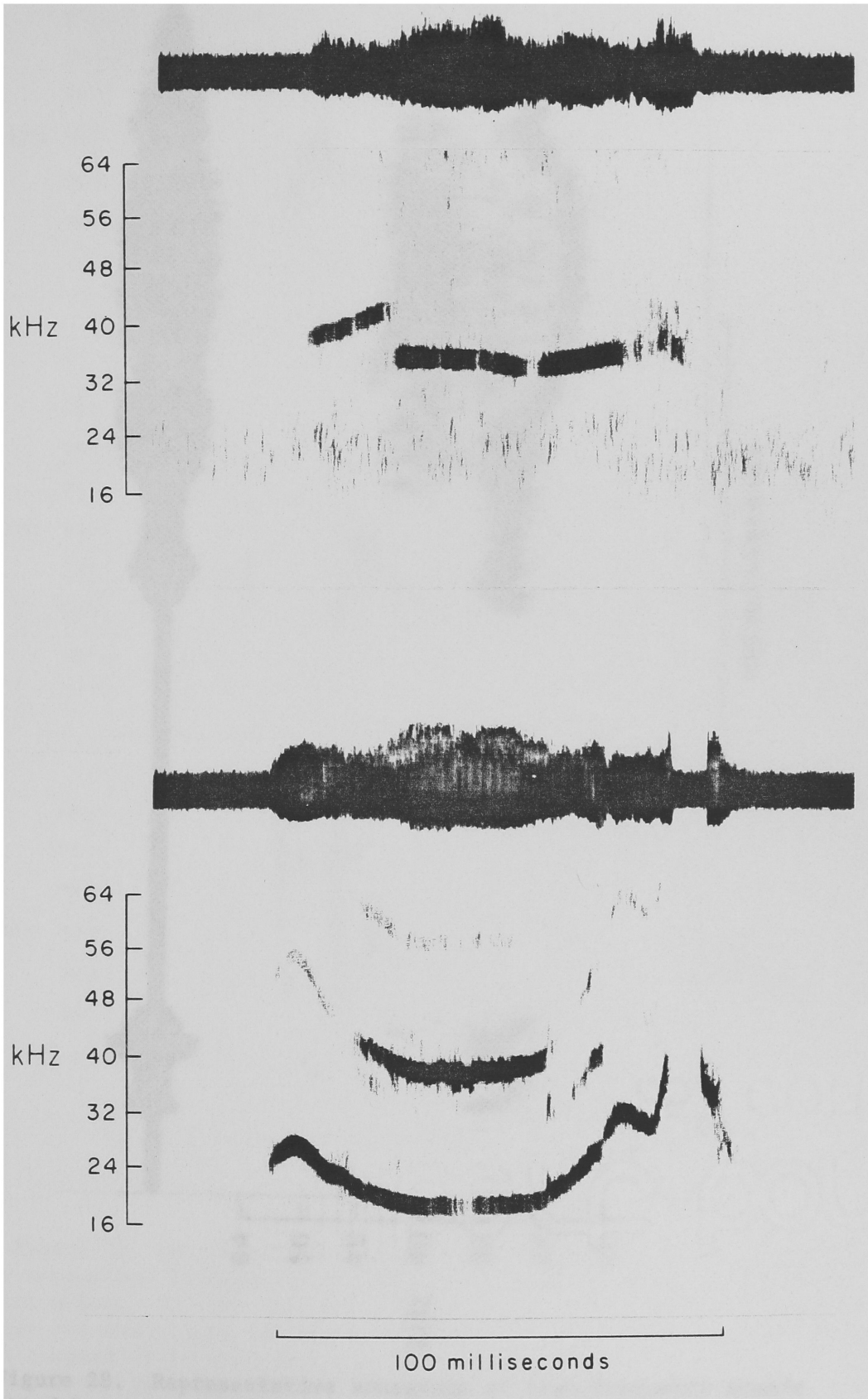


Figure 27. Representative sonagrams of high-frequency sounds emitted by estrous female hamsters following brief exposures to males. See legend of Figure 25 for explanation.

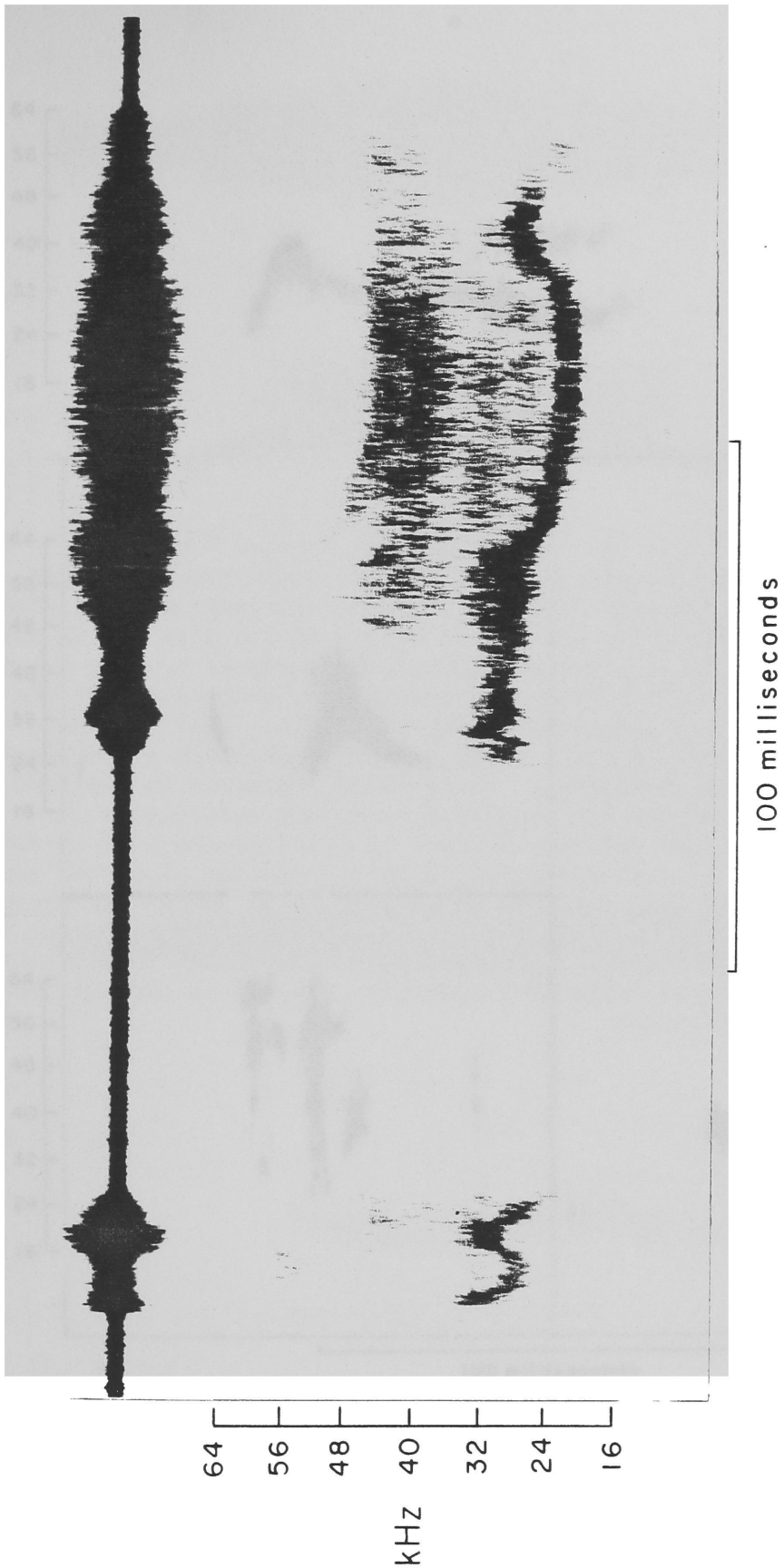


Figure 28. Representative sonagrams of high-frequency sounds emitted by estrous female hamsters following brief exposures to males. See legend of Figure 25 for explanation.

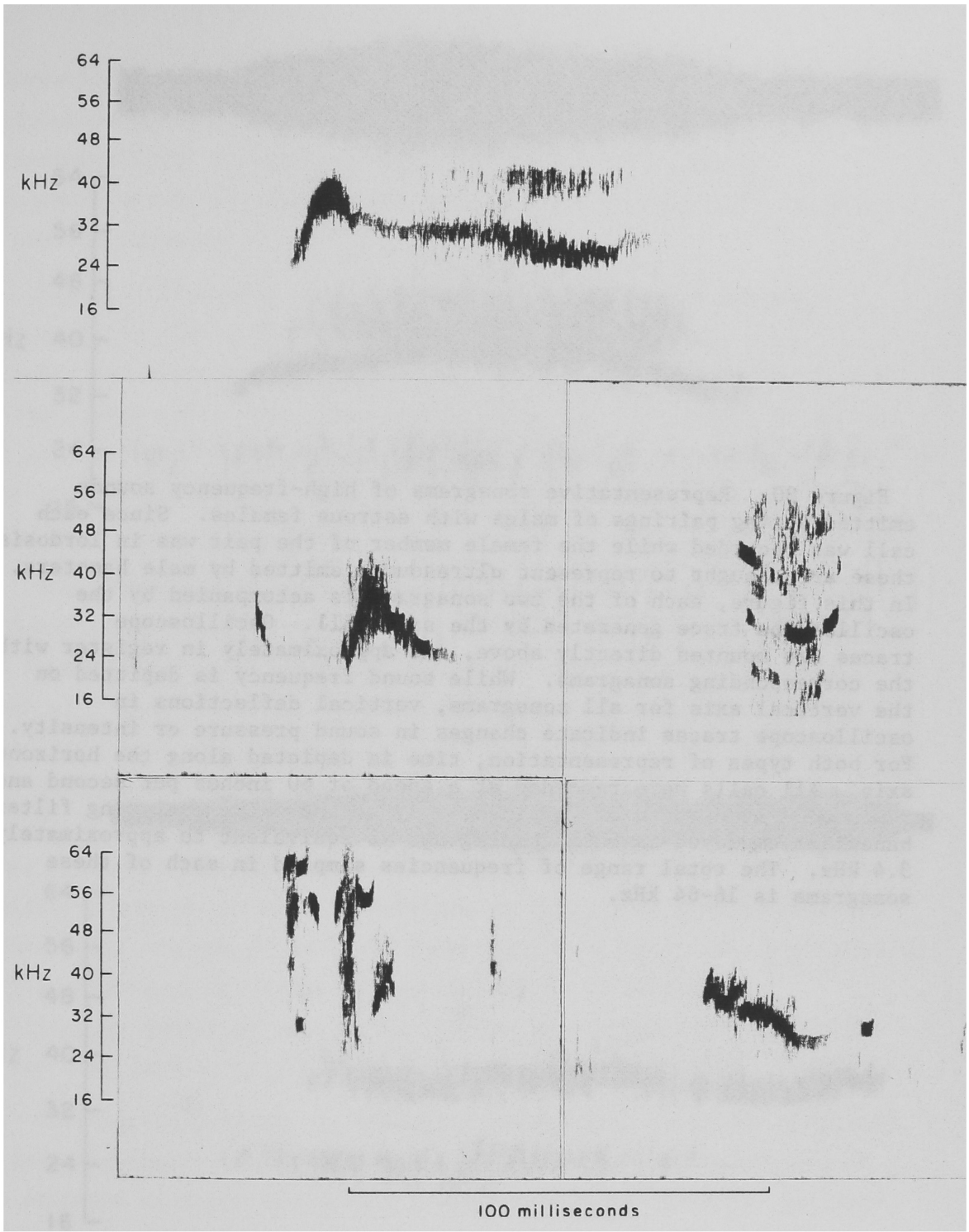
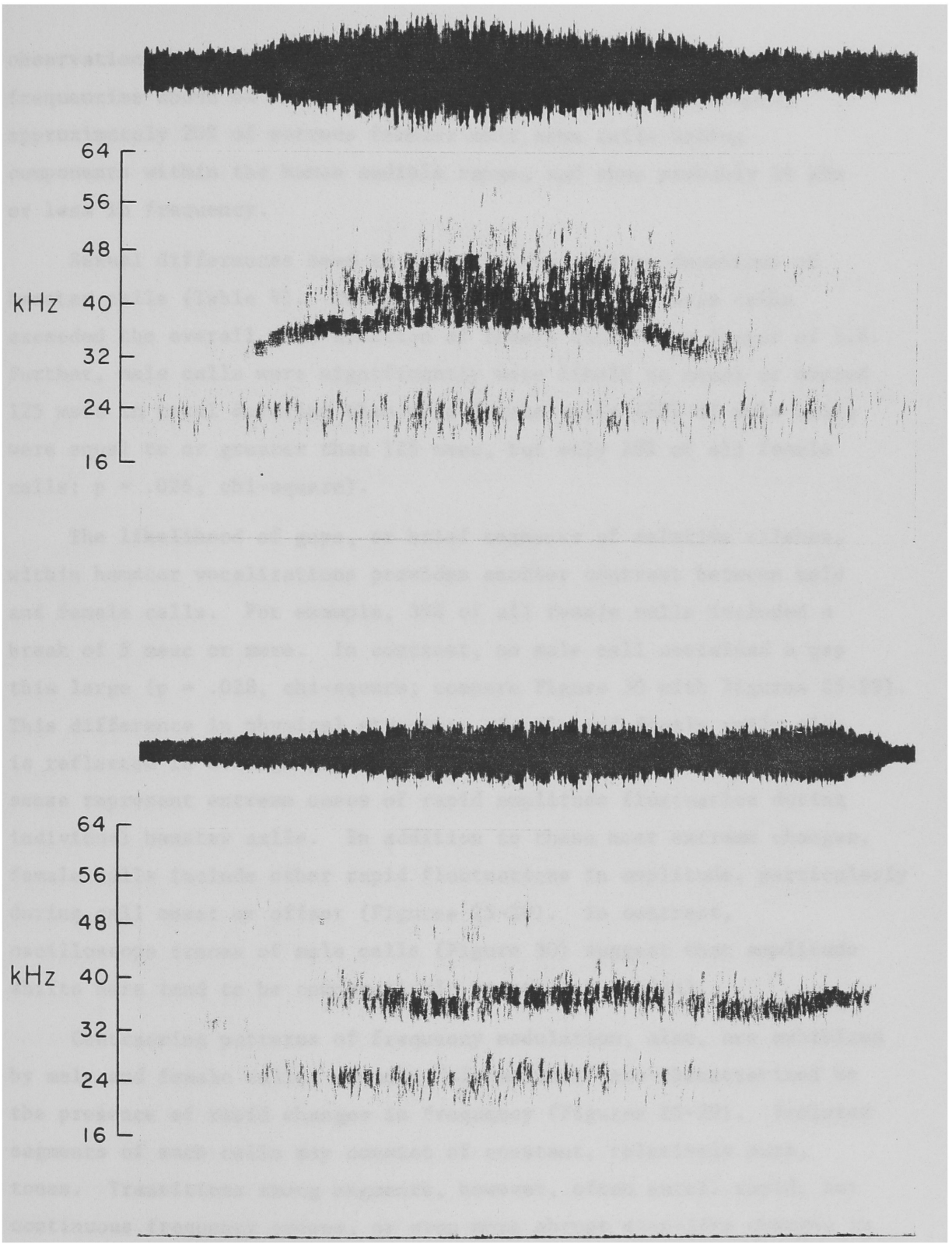


Figure 29. Representative sonagrams of high-frequency sounds emitted by nonestrous females and by estrous females prior to any contact with males. In each sonagram, frequency is depicted on the vertical axis and time along the horizontal axis. All five calls were recorded at a speed of 60 inches per second and reproduced at a speed of 7.5 inches per second. The analyzing filter bandwidth employed in making sonagrams is equivalent to approximately 3.4 kHz. The total range of frequencies sampled in these sonagrams is 16-64 kHz.

Figure 30. Representative sonagrams of high-frequency sounds emitted during pairings of males with estrous females. Since each call was recorded while the female member of the pair was in lordosis, these are thought to represent ultrasounds emitted by male hamsters. In this figure, each of the two sonagrams is accompanied by the oscilloscope trace generated by the same call. Oscilloscope traces are mounted directly above, and approximately in register with, the corresponding sonagrams. While sound frequency is depicted on the vertical axis for all sonagrams, vertical deflections in oscilloscope traces indicate changes in sound pressure or intensity. For both types of representation, time is depicted along the horizontal axis. All calls were recorded at a speed of 60 inches per second and reproduced at a speed of 7.5 inches per second. The analyzing filter bandwidth employed in making sonagrams is equivalent to approximately 3.4 kHz. The total range of frequencies sampled in each of these sonagrams is 16-64 kHz.


64
56
48
kHz 40
32
24
16
64
56
48
kHz 40
32
24
16
100 milliseconds

observations have revealed a small fraction of calls including frequencies above 64 kHz. Conversely, as mentioned previously, approximately 20% of estrous females emit some calls having components within the human audible range, and thus probably 16 kHz or less in frequency.

Sexual differences seem to exist in the average durations of hamster calls (Table V). The mean duration of these male calls exceeded the overall mean duration of female calls by a factor of 1.6. Further, male calls were significantly more likely to equal or exceed 125 msec in total duration than were female calls (82% of male calls were equal to or greater than 125 msec, but only 28% of all female calls; $p = .026$, chi-square).

The likelihood of gaps, or brief segments of relative silence, within hamster vocalizations provides another contrast between male and female calls. For example, 39% of all female calls included a break of 5 msec or more. In contrast, no male call contained a gap this large ($p = .028$, chi-square; compare Figure 30 with Figures 25-29). This difference in physical structure of male and female calls also is reflected in average gap durations (Table V). These gaps in one sense represent extreme cases of rapid amplitude fluctuation during individual hamster calls. In addition to these most extreme changes, female calls include other rapid fluctuations in amplitude, particularly during call onset or offset (Figures 25-29). In contrast, oscilloscope traces of male calls (Figure 30) suggest that amplitude shifts here tend to be comparatively smooth and gradual.

Contrasting patterns of frequency modulation, also, are exhibited by male and female calls. Female "ultrasounds" are characterized by the presence of rapid changes in frequency (Figures 25-29). Isolated segments of such calls may consist of constant, relatively pure, tones. Transitions among segments, however, often entail rapid, but continuous frequency sweeps, or even more abrupt step-like changes in frequency. In contrast, male calls tend to be quite constant in frequency, or to involve only gradual and continuous transitions in

frequency (Figure 30). Thus, while 70% of all female calls included at least one frequency change of at least 5 kHz within 10 msec, only 18% of male calls included a frequency transition of comparable rapidity ($p = .003$, chi-square; also see Table V).

Discussion

Physical parameters that we have ascribed to hamster ultrasounds are similar to those observed by Sales (1972a; also, Sewell, 1970; and Sales, 1972b). In particular, average "dominant" frequencies and call durations summarized in Table V fall well within the corresponding ranges cited by Sales (1972a).

We have described patterns of amplitude and frequency modulation correlated with different test situations and female hormonal state, and with differences in the sex of the sound source. Early informal impressions had suggested that the physical parameters of calls emitted by estrous females following brief exposure to a male might differ consistently from those characteristic of females of other hormonal states or in other experimental situations. Data summarized in Table V fail to provide convincing support for such a distinction. Nor did direct comparisons of the post-male calls emitted by estrous females in the presence versus absence of imitation calls yield dramatic or consistent differences. More extensive comparisons of female calls produced in different contexts may still uncover such differences. Based on the present data, however, high-frequency sounds emitted by female hamsters in a variety of situations are quite similar. In contrast, these female calls seem distinctive in some of their physical characteristics from calls emitted by an individual male hamster (Table V). In particular, high-frequency sounds produced by females seemed more complex than those emitted by a male. For example, female calls were more likely to include rapid fluctuations in amplitude and frequency (Table V). In general, signals which include rapid changes in frequency or amplitude are well adapted to facilitate the localization of a sound source (Marler, 1961). Thus, the differences in call structure mentioned above are consistent with the operation of female calls over greater distances, and for different purposes, than the male calls included in our sample.

Observations subsequent to those detailed in this thesis (Floody & Pfaff, 1974) confirmed that male ultrasounds, in general, contain significantly fewer rapid changes in amplitude and frequency than ultrasounds by females. In addition, this study showed that male call structures depend upon contextual factors. Calls produced by males in the presence of estrous females tend to have lower minimum frequencies, higher maximum frequencies, longer durations and fewer rapid frequency changes than calls by solitary males. These results, together with those summarized previously in this chapter, show that both sex differences and situational factors influence the structures of hamster ultrasounds. The frequency and amplitude changes typical of calls by females and solitary males should facilitate the localization of a calling individual. Thus, these calls could serve as social communications over moderate distances. Calls by males in the presence of females would be more difficult to localize and might operate over shorter distances to serve a different social function.

GENERAL DISCUSSION

A concern for acoustic communication among golden hamsters grows naturally from the unremitting aggression exhibited by hamsters of both sexes and most endocrine states (Chapters II-III). That is, the extreme aggressiveness of male and female hamsters seems most consistent with a social organization in which individuals of both sexes live solitarily. As a consequence of this extreme social intolerance, individual hamsters might be separated by considerable distances. The maintenance of such a social organization might then depend upon communications which announce the state of receptivity and aggressiveness of a female without requiring close physical contact with her. Such noncontact signals might function most economically by attracting potential mates on a female's estrous day. In this regard, high-frequency sounds emitted by estrous female hamsters seem well suited, in overall intensity and in patterns of amplitude and frequency modulation, to serve as sexual advertisements over moderate distances (also see Sales, 1972a). In conjunction with long-distance olfactory cues, these calls would indicate both the location and

reproductive state of an individual female (Chapter VI). In such a manner, these signals could perform integral roles in the coordination of important social behaviors (male-female interaction leading to copulation) with reproductive (endocrine) state. Male calls also could participate in this process by affecting female sexual behavior at closer range, or within the confines of a burrow system.

CHAPTER VI. DETERMINANTS OF ULTRASOUND PRODUCTION BY FEMALE AND MALE HAMSTERS

Determination of the communicatory value of any natural sound should include consideration of the stimuli typically associated with changes in the rate of emission of that signal (e.g., Bell, 1974). This chapter summarizes the results of studies of hormonal and sensory determinants of hamster ultrasound production.

GENERAL METHOD

Subjects and Maintenance

Random-bred male and female hamsters, of the LVG:LAK strain, were purchased from Lakeview Hamster Colony, Newfield, New Jersey. Individuals were 28-63 days old when acquired and each was housed separately in a metal cage of dimensions 25 x 18 x 18 cm or 30 x 30 x 18 cm. Purina rat chow and tap water were supplied ad libitum. Cabbage, sunflower seeds and Purina guinea pig chow were supplied at weekly intervals.

Lighting in the colony rooms varied according to a reversed 12-hour bright:12-hour dim cycle. Temperature fluctuations were attenuated, ranging normally between 20 and 25 degrees centigrade, with extremes of 12 and 32 degrees over a period exceeding two years.

Tests for the emission of high-frequency sounds were conducted in 30 x 30 x 18 cm metal cages or in 48 x 27 x 20 cm polycarbonate cages. At the time of participation in experiments, hamsters ranged between 135 and 385 days of age. All individuals were intact, and all females maintained regular 4-day estrous cycles (Orsini, 1961) throughout testing.

The Detection of High-Frequency Hamster Sounds

An ultrasonic detector produced by Holgates of Totton, Southhampton, England (Holgate ultrasonic receiver Mk. V) was used routinely for the detection of high-frequency hamster vocalizations. This type of instrument translates high-frequency signals into audible sounds that may be counted directly by an observer. The sensitivity of our Holgate receivers was increased considerably as a consequence of modification

of the pre-amplifier stage by M. Rossetto of The Rockefeller University (circuit diagrams available directly from M. Rossetto or through the author). Quantitative estimates of the acoustic thresholds of these particular instruments were conducted as detailed below.

Hamster calls emphasize frequencies within the range of 20-45 kHz (Chapter V). The mean "dominant frequency" of calls observed spectrographically in our previous study was 35 kHz. Further, this average seems highly representative of mean dominant frequencies for groups including only estrous females, nonestrous females, or males. Accordingly, of the calls sampled, each had some component within the frequency range of 20-45 kHz, and more than 90% exhibited dominant frequencies within this range.

Holgate receivers are relatively narrow-band instruments. Thus, sensitivity decreases with increasing frequency differences between an input signal and the frequency to which the detector is tuned. To achieve an optimal match between peak Holgate sensitivity and the average dominant frequency characteristic of hamster calls, our Holgate receiver was tuned to a frequency of 35 kHz. Since signal amplification also was maintained at a constant level in all experiments, it was possible to estimate the effective frequency bandwidth of our detection device under conditions similar to those encountered during behavioral tests (Experiments 7-14). Holgate sensitivity was determined with reference to a calibrated condenser microphone manufactured by Bruel and Kjaer of Naerum, Copenhagen, Denmark (a B & K 1/4-inch condenser microphone, type 4135, used in conjunction with a B & K cathode follower, type 2615, and a B & K microphone amplifier, type 2604). Constant tones of known frequency, amplitude and wave form were generated by a system described previously (Chapter V). The following elements were inserted into this system to allow the generation of tone-pulses of known duration and repetition rate: an American Electronics Laboratories laboratory stimulator (model 104A) and a Grason-Stadler electronic switch (model 829C). Generated signals were monitored visually on a Tektronix, Inc. dual-beam oscilloscope, type 502A. In particular, 100 msec. pulses of

variable frequency were programmed with repetition rates of 1-2 per second. A decade attenuator allowed the amplitude of these signals to be changed in convenient, known increments. Pulses of appropriate frequency and amplitude produced clearly audible sounds when monitored with a Holgate receiver. With the microphone of such a receiver approximately 1 meter from the Kuhl-type speaker (see Kuhl et al., 1954) which comprised the final output stage of the above system, the amplitude of the programmed signal was altered systematically until it became impossible for an observer to distinguish audible counterparts of the signals from internal noise generated by the Holgate receiver. This value of the amplitude (in dB SPL re. 2×10^{-4} dyne/cm^2) was defined as the Holgate threshold for the frequency to which it was tuned and for the frequency of the signal being detected.

With the Holgate receiver used in these experiments tuned to 35 kHz, the "Holgate thresholds" for signals of various frequencies were determined as described above. Since hamster ultrasounds are quite variable in frequency (Chapter V), the primary concern here was simply to insure that our detection device was not missing all signals outside of a very restricted frequency range, and thus presenting a distorted picture of basal call rates and the effects of various stimuli on the rate of signal emission by hamsters. The results of our threshold determinations indicated that the Holgate receiver used here was very sensitive indeed to signals near its tuned frequency of 35 kHz. Input signals of 35 kHz were associated with thresholds of 19-23 dB SPL. Sensitivity was less at other frequencies. However, frequencies throughout the frequency range of 20-45 kHz were associated with thresholds of 40 dB SPL or less.

Threshold determinations described above were conducted under virtually ideal conditions in which extraneous high-frequency noise was negligible. Most observations of high-frequency sounds emitted by hamsters (Experiments 7-14) were conducted under conditions in which extraneous noise and interference were more prominent. Thus, it is impossible to estimate very exactly the fractions of hamster ultrasounds

detected and missed by our procedures. However, earlier estimates of average call intensity (Chapter V) suggest that hamster "ultrasounds" equal or exceed 50 dB SPL in intensity at distances (35-50 cm) well beyond those at which microphones were positioned during these experimental observations (roughly 5-15 cm). Further, we have seen that most hamster ultrasounds do include at least some components falling within the frequency range of 20-45 kHz and that frequencies near 35 kHz typically receive particular emphasis. Finally, the physical structures of most high-frequency sounds emitted by hamsters emphasize constant-frequency segments of variable duration. Constant-frequency signals are well suited to detection by instruments operating according to heterodyne principles, such as Holgate receivers. From all of these considerations, we conclude that the instruments and procedures used here for the simple detection of hamster ultrasounds are appropriate to the physical characteristics of those signals and probably do detect nearly all hamster calls emitted in situations such as those emphasized in subsequent experiments.

Test Procedures

An experimental paradigm which has proven useful in studying the stimulus control of hamster high-frequency sounds has revolved around the brief presentation of a test stimulus to a female (Experiments 7-12) or male (Experiments 13-14) and the comparison of rates of calling observed during test segments (a) prior to the introduction of the test stimulus (3-5 minutes), (b) while the stimulus was present (30 seconds - 5 minutes), and, (c) following removal of the test stimulus (3-10 minutes). Further, immediately prior to the pre-stimulus segment of a test, each subject was exposed to ("familiarized with") the test cage, and other aspects of the experimental situation (e.g., the large electrically-shielded chamber in which test cages were placed during tests), for a time roughly equal to the duration of an entire test (the sum of test segments a-c above). Pre-test familiarization usually was conducted in the same quiet, dimly-lit room used for testing, sometimes during testing of a different experimental subject. During each observation period included in a test (a-c above), the microphone of a Holgate ultrasonic receiver was held as close as possible (5-15 cm) to the subject and the Holgate's audible output was

monitored for the occurrence of high-frequency (20-45 kHz, see previous section) calls. Throughout most tests, the frequency of calling was noted during successive 1-minute intervals. The exact durations of individual test segments, and the identity of test stimuli presented during each of Experiments 7-14 are summarized in Table VI. Minor departures from this basic pattern will be discussed subsequently in connection with each individual experiment.

Table VI: Routine Test Procedures

			Durations of Test Segments		
Experiment #	Subject	Stimulus	Pre-Stimulus (min.)	Stimulus (sec.)	Post-Stimulus (min.)
7	Female	Awake Male	3	30	3
8	Female	Awake Male	5	30	10
9	Female	Awake Male	5	30-60	5, + time in lordosis
10	Female	Male-Shavings	3	30	3
11	Female	Anesthetized Male	5	300 (5 min.)	5
12	Female	Anesthetized Male	5	60	5
13	Male	Awake Female	5	60	5
14	Male	Anesthetized Female	5	60	5

Statistics

Most statistical analyses involved comparisons of average scores contributed by the same individuals during different conditions: i.e., segments of a test, at different stages of the female estrous cycle, or in response to different test stimuli. Thus, our experimental designs have generally permitted each individual to serve as its own control.

The particular statistical tests employed in these comparisons were the sign-test (Siegel, 1956) and the t-test for dependent means (Edwards, 1966). All statements regarding statistical significance and the chance probabilities of observed differences have been based on two-tailed tests of significance.

EXPERIMENT 7: VARIATIONS IN FEMALE VOCALIZATION RATE WITH THE ESTROUS CYCLE

During preliminary observations, we found that approximately 20% of intact adult female hamsters in our colony emitted distinctive audible vocalizations. These calls appeared almost exclusively on estrous day, while test females were sexually receptive. This correlation suggested that these vocalizations might function primarily in social communication related to reproduction (see also Sales, 1972a). Consistent with this role in social communication were results of other informal experiments indicating that brief exposure to a male, or to male odors, could facilitate calling by a receptive female. For example, one estrous female, exposed to clean cedar shavings in her home cage, failed to vocalize during a ten-minute test. However, during the immediately succeeding ten minutes, the female was exposed to shavings from the home cage of a male and 20 vocalizations were recorded. Finally, during a ten-minute test following a 30-second exposure to a male, the same female emitted 146 "estrous vocalizations."

The physical characteristics of audible vocalizations emitted by female hamsters suggested that high-frequency components might be present in these calls. Indeed, noting the relatively small fraction of females ever observed to emit audible cries, we suspected that these calls might themselves represent only that small fraction of high frequency calls which included components within the human audible range. This speculation was encouraged initially by informal observations (and later by experiments summarized in Chapter V) indicating that all of our colony females did emit some ultrasonic calls, even individuals never observed to emit audible vocalizations. Clearly, the next step in the analysis of stimulus determinants of vocal communication among hamsters was the examination of variations in female ultrasound rate with the estrous cycle, and following brief male-female contact.

Method

Subjects and Test Procedures

Each of 10 naive female hamsters participated in 9-10 tests covering at least two complete estrous cycles. That is, females experienced at least 2 tests on each of the four days of the normal estrous cycle. Successive test-days were separated by intervals of 1-4 days.

A single adult male served as the experimental stimulus in all tests. While each female was tested in her own home cage, tests were preceded by 7-10 minute periods of familiarization to the general experimental situation (see General Method). Subsequently, a Holgate ultrasonic receiver (tuned to 35 kHz) was used to monitor the high-frequency sounds produced by a female during a 3-minute period prior to the introduction of an awake male (see Table VI). The stimulus male then was placed in the female's home cage and the pair was observed during 30-seconds of free social interaction. Finally, the female was observed for an additional 3-minute period following the removal of the male. As in all experiments of this general design, the timing for the post-male period was begun when the female first emerged from lordosis, if this posture was maintained at all following the male's removal.

Observations discussed here as Experiment 7 actually formed only part of a more extensive series of tests which included observations of the same 10 females before, during and after exposure to soiled shavings from the home cage of the same stimulus male. However, the two segments of testing were separated by 4-6 weeks and equal groups of 5 females experienced each of the two possible orders of test series (male then shavings or shavings then male). As the two series of tests seemed not to interact in any way, results from the series on which shavings were presented will be reserved for a later section and will be discussed as a separate experiment (see Experiment 10).

Results

Results summarized in Table VII document a clear correlation between rates of "ultrasound" emission and endocrine state. During

Table VII: Mean Rates of Ultrasound Emission Before, During and After Brief Exposure to an Awake Male. Variations throughout the Estrous Cycle.

Cycle Day	Mean Ultrasounds/Minute (SEM*) Pre-Male	Male Present	Post-Male
1 (Estrous)	2.47 (0.79)	10.10 (1.61)	3.03 (0.65)
2	0.25 (0.13)	8.73 (1.68)	0.52 (0.25)
3	0.21 (0.12)	6.63 (1.18)	0.32 (0.20)
4	0.58 (0.22)	6.05 (1.09)	0.24 (0.15)

*Standard error of the mean

test segments prior to (pre-male) and following (post-male) exposure to an awake male, rates of high-frequency calling by females were significantly greater on estrous day ("Day 1") than on any of the 3 nonestrous days of the cycle ($p \leqslant .04$, sign test). Differences among days 2-4 of the cycle were inconsistent and statistically unreliable.

In contrast to very clear trends during pre- and post-male segments of tests, much less cyclicity in calling frequency is apparent in the presence of a male (Table VII). Ultrasound rates on a test female's Day 1 exceeded those on Day 4 by a difference which approaches statistical significance ($p = .052$, t-test). However, this trend is not supported in statistical evaluations of observed differences

between rates on Day 1 and those on either of the other two non-estrous days of the female cycle ($p \gtrsim .13$, t-test). Further, differences among days 2-4 again were inconsistent and statistically unreliable.

Within tests conducted on any day of the female estrous cycle, rates of ultrasound emission during male-female interaction consistently exceeded those pre- and post-male ($p \lesssim .022$, sign test, see Table VII). In contrast, direct comparisons of rates of female calling before and after brief social contact failed to reveal a consistent difference on estrous day ($p = .35$, t-test), though differences observed at other points during the female cycle did approach significance ($p = .068$ for scores on Day 2, $p = .082$ for scores on Day 4, t-tests).

Discussion

Results summarized in Table VII indicate clearly that female hamster "ultrasounds" encode information regarding reproductive state (as well as location). Basal (pre-male) rates of high-frequency sound emission by estrous females greatly exceed those typical of non-estrous females. Both pre- and post-male, females emit high-frequency calls most frequently when they are most sexually receptive. This relationship is highly consistent with the interpretation of female hamster "ultrasounds" as sexual advertisements (Sales, 1972a; Chapter V).

The interpretation of high-frequency sounds observed during male-female contact ("male-present" period in Table VII) is difficult. Hamsters of both sexes emit ultrasounds, and those observed during heterosexual pairings often cannot be attributed with certainty to either partner. Results of a later experiment (see Experiment 9) suggest very strongly that female hamsters do not emit high-frequency "estrous calls" while actually in the lordosis posture. Since estrous females may spend most of their periods with males in lordosis, high rates of calling during such pairings may be attributable largely to the males. Similarly, high and relatively constant rates of calling during "male-present" periods throughout the female estrous cycle may reflect indiscriminate calling by the male rather than (or in addition

to, see Experiments 10-12) some increase in a female's rate of calling while in the presence of a male. Observations of the sexual and agonistic behaviors of male hamsters in heterosexual encounters suggest that a male's interest in and pursuit of a female does not differ greatly as a function of her estrous state (unpublished observations, also see Payne & Swanson, 1970).

Data summarized in Table VII include few consistent differences between the rates of ultrasound emission before and after the presentation of a male. In particular, rates of calling pre- and post-male on estrous day were quite similar (2.47 and 3.03 calls/minute, respectively). This finding contrasted vividly with some very early observations of audible cries emitted by estrous female hamsters. At least some such informal experiments included dramatic examples in which brief exposure to a male caused a drastic elevation in the rate of calling by a female, and in which this facilitation of calling persisted well beyond the time of the male's removal (see the example cited in the introduction to this experiment). A subsequent experiment (Experiment 8) investigating fluctuations in "ultrasound" rate with the estrous cycle focused special attention on several factors possibly involved in the production of a difference in rates of calling pre- versus post-male.

EXPERIMENT 8: VARIATIONS IN FEMALE VOCALIZATION RATE WITH THE ESTROUS CYCLE AND FOLLOWING EXPOSURE TO AN AWAKE MALE

Initial speculation as to possible explanations for the absence of a pronounced pre-post (pre-male versus post-male) difference in Experiment 7 (as opposed to earlier informal results) focused on three sorts of variables: (a) test location; (b) the durations of observation periods; and (c) the degree of social experience gained by subjects in the course of an experiment.

(a) Some informal observations of the audible calls emitted by estrous females had been conducted in areas or cages which were familiar to the female but did not coincide with her home or nest area. Therefore, Experiment 8 included two series of tests which differed with respect to test location. One series was conducted, as before, in

the individual female's home cage. The other occurred in a cage other than the home cage, but to which the female was exposed for at least 15 minutes immediately before each test. A particular test cage was assigned to each participant and was not cleaned in the course of a test series, thus insuring considerable familiarity with these non-home cages.

(b) During earlier informal observations, females often were subjected to continuous observation for 10-15 minutes, during which calls appeared in distinct bursts separated by relatively prolonged periods of silence. It seemed possible that post-male test segments in Experiment 7 (3 minutes) were too brief to provide an adequate sample of calls to permit determination of the average rate of vocalization following male-female contact. A more extensive sampling of post-male calls in Experiment 8 was insured simply by increasing the duration of this test segment from 3 to 10 minutes. The duration of pre-male observation also was increased, from 3 to 5 minutes.

(c) While initially naive, participants in Experiment 7 experienced an extensive and very concentrated series of experimental tests including many brief social interactions with males. Under these circumstances, it seemed possible that short-term changes in pre- or post-male rates of vocalization could have occurred, and that these changes could have obscured any pre-post differences. Here, we were concerned not with the total amount of social or experimental experience accumulated by a subject, but, instead, with the rate at which this experience was gained and its strictly <u>short-term</u> effects. To counteract such changes, participants in Experiment 8 were tested on only two cycle days (Days 1 and 3) instead of all four, thus decreasing both the total amount of experimental experience gained (4-5 tests per female as opposed to 9-10 in Experiment 7) and the rate at which this experience was accumulated (e.g., clearly no tests in Experiment 8 could be conducted on successive days). On the other hand, to facilitate the evaluation of effects of these variables (especially a-b above) on our results, the eight females participating in Experiment 8 were selected randomly from the ten participants in Experiment 7. An interval of three weeks was inserted between the last test in Experiment 7 and the first in

Experiment 8 to minimize any effects due to possible long-term changes in vocalization rate. Direct comparisons of average pre-male vocalization rates during comparable 3-minute segments from home-cage tests of females participating in both experiments (1.8 calls/minute in Experiment 7 as opposed to 1.9/minute in Experiment 8) suggest that relatively long-term changes in call rate did not affect any differences in the results obtained in these two experiments.

Method

Subjects and Test Procedures

Most details regarding subjects and procedures employed in this experiment have been discussed above (also see Table VI). Each of eight intact females experienced two series of tests, one in her home cage and the other in a familiar, but non-home, cage. Each test series included 2-3 tests on estrous day (Day 1) and 2-3 tests on a day near the midpoint of the nonestrous phase of the cycle (Day 3). The two test series were counterbalanced for order. Each individual test included 5 minutes of observation pre-male, 30 seconds of exposure to a particular stimulus male (the same male for all females, and the same individual used as a stimulus male in Experiment 7), and 10 minutes of observation following removal of the male. As in the case of Experiment 7, the timing for the 10 minute post-male period was begun when the female first emerged from lordosis, if this posture was maintained at all following the male's removal.

Results and Disucssion

Results summarized in Table VIII indicate that the type of cage (home or familiar) in which tests were conducted did not exert any consistent effect on rate of ultrasound emission during any test segment ($p \gtrsim .11$ for all home cage versus familiar cage comparisons involving comparable test segments and cycle days, t-test). Further, direct comparisons of pre- and post-male vocalization rates failed to reveal any consistent differences regardless of the caging condition considered (home, familiar or the combination of the two groups of data). Thus, our manipulation of test cages has not proved an important variable affecting the appearance of a pre-post difference in female ultrasound rate.

Table VIII: Mean Rates of High-Frequency Calling Before, During and After Brief Exposure to an Awake Male. Comparison of Rates in Home and Familiar Cages. Differences Between Days 1 and 3 of the Estrous Cycle.

		Mean Ultrasounds/Minute (SEM)		
Cycle Day	Cage Condition	Pre-Male	Male Present	Post-Male
1	Home	1.80 (0.70)	11.21 (1.23)	2.09 (0.24)
	Familiar	2.33 (0.83)	12.79 (1.39)	3.07 (0.46)
	Combined	2.06 (0.42)	12.00 (0.64)	2.58 (0.25)
3	Home	0.15 (0.11)	12.33 (2.05)	0.10 (0.06)
	Familiar	0.17 (0.11)	15.75 (2.53)	0.09 (0.03)
	Combined	0.17 (0.07)	14.04 (1.34)	0.09 (0.03)

Comparisons of "combined results" included in Table VIII reveal trends virtually identical to those summarized in Table VII. For example, female calling before and after brief social contact was most frequent when the female was sexually receptive (Day 1), and rates then exceeded those at the midpoint of the nonestrous phase (Day 3) by factors of at least 12 ($p = .008$ both pre- and post-male, sign test). Similarly, rates of vocalization by male-female pairs consistently exceeded those characteristic of isolated females before or after brief social contact ($p = .008$ for all comparisons of male-present periods with pre- and post-male segments on either Day 1 or Day 3, sign test). Further, rates of calling during the male-present segments of tests again failed to exhibit consistent variations with the estrous cycle. As before, high rates of calling here probably reflect some emission of high-frequency sounds by the stimulus male, as well as by test females.

Extensive similarities among trends summarized in Tables VII and VIII suggest that our manipulations of (a) test location (home versus familiar cages), (b) durations of pre- and post-male test segments, and, (c) degree of short-term experimental or social experience, have failed to affect the relative rates of calling by female hamsters before and after brief exposure to an awake male. In the case of (a) above, this impression is strengthened by results of direct comparisons of "ultrasound" rates in home versus familiar cages. Similarly, in further evaluating influences of factor (b), we have examined temporal variations in call rate within pre- and post-male test segments on a minute-by-minute basis. We have not found any fraction of the 10-minute post-male period which consistently was associated with higher rates of calling than those seen during pre-male test segments. We conclude that any existing temporal variations in call rate are inappropriate to account for the failure of pre-post differences obvious in pilot experiments to appear in average data from Experiments 7 and 8.

Successive tests in the series of 4 or 5 summarized in Table VIII must have been associated with differences in the degrees of short-term experience previously accumulated by experimental subjects. Therefore, it seemed possible that a finer analysis of the effects of short-term experience (factor c above) might be achieved by focusing attention on the first Day 1 test in the experimental series. Indeed, we found that post-male scores on the first estrous test experienced by each female did reliably exceed the rate of calling observed in the pre-male segment of the same test (Table IX). On subsequent Day 1 tests, the rate of

Table IX: Mean Rates of Ultrasound Emission Before and After Brief Exposure to an Awake Male. The First Estrous-Day Test Experienced by Each Female.

Mean Ultrasounds/Minute (SEM)	
Pre-Male	Post-Male
0.63 (0.49)	2.93 (0.74)
$p = .016$ (sign test)	

ultrasound emission during the pre-male segment increased significantly ($p = .006$, t-test) and approximated levels of calling exhibited by the same females in the post-male test segment. These results suggested that, at least under some circumstances, rates of ultrasound emission following a brief exposure to a male did reliably exceed "spontaneous" rates observed prior to heterosexual social contact. This suggestion is highly consistent with the results observed in pilot experiments during which only audible calls were monitored. Further, these results suggested that rates of calling during some test segments tended to increase with time or over successive tests. Such a "priming" effect could have stemmed from learning (e.g., classical conditioning), or from the simple accumulation of, and response to, male odors left over from previous encounters in the test series. A subsequent experiment (Experiment 9) attempted to specify the relative contributions of these two factors to "priming" effects and pre-post (pre-male versus post-male) differences in female vocalization rates.

EXPERIMENT 9: OLFACTORY CUES IN "PRIMING" EFFECTS AND "PRE-POST" RATES OF FEMALE VOCALIZATION

The present experiment was conducted in an attempt to determine if a gradual build-up of olfactory cues originating with the stimulus male might have accounted for the appearance of a "priming" effect on pre-male call rates, and the associated absence of an expected difference in ultrasound rates pre- versus post-male. Specifically, we hypothesized that thorough washing of male odors from the test cages before pre-male testing should result in the absence of any short-term "priming" of vocal responses, and the appearance of a significant pre-post difference in female ultrasound rate.

Method

Subjects and Test Procedures

Most aspects of the design of this experiment were very similar to those of previous experiments in this series (see Table VI). Twelve female hamsters were observed on 3 estrous days within a period of 12 days. Six females were naive, while the remaining six had participated

previously in another experiment included in this report. However, naive and experienced subjects did not differ in average rates of high-frequency vocalization during any segment of tests described here. Therefore, their scores have been combined in all subsequent analyses (see Results).

Females were monitored for emission of "ultrasounds" during a 5-minute pre-male period which followed a period of at least 15 minutes of familiarization to the test cage. At the end of the initial 5-minute test segment, a male was introduced and allowed to remain in the test cage for 30-60 seconds. In this experiment, each female was randomly assigned a different male at the beginning of the test series and these pairings remained constant throughout subsequent tests. Females almost invariably exhibited lordosis in the presence of the male and this posture was maintained for variable periods of time (mean = 39 sec.; range = 0 - 345 sec.) following the male's removal. High-frequency sounds were monitored continuously throughout these periods. Finally, each female was observed for an additional 5-minute post-male period which commenced at the instant of emergence from lordosis.

The principal feature distinguishing this experiment from those summarized in Tables VII-IX concerns the nature of the test cage. Here, cages were rinsed thoroughly in hot water after each test. New shavings were introduced prior to each test in an additional effort to maintain the olfactory environment at a constant level throughout the test series, as free as possible from any male odors.

A more minor procedural detail distinguishing this from previous experiments concerned the location of pre-test familiarization to the test cage. In previous experiments, this familiarization was conducted in the test room, often within several meters of the female engaged in the immediately preceding test. In order to reduce any auditory (or olfactory) cues received by a female during these periods, familiarization sessions for the present experiment were conducted in a room adjacent to the test room, so that the test cage containing the familiarized female had to be moved several meters only 1-2 minutes prior to the initiation of the 5-minute pre-male segment of testing. The fact that

rates of ultrasound emission during successive 60-second intervals of the pre-male period did not increase (actually they decreased significantly between the first and fifth minutes of pre-male testing, $p < .006$, sign test) indicates that this minor manipulation did not depress rates of calling during the pre-male period and could not have caused a spurious difference between this and the post-male segment of testing.

Results

Results summarized in Table X show that a reduction in the strength

Table X: Mean Rates of Ultrasound Emission Before, During and After Brief Exposure to an Awake Male. Variations Over Successive Estrous-Day Tests.

	Mean Ultrasounds/Minute (SEM)			
			Post-Male	
Test #	Pre-Male	Male Present	Female In Lordosis	Female Out of Lordosis
1	0.22 (0.11)	5.00 (1.62)	0 (0)	4.00 (0.92)
2	0.72 (0.45)	6.75 (2.17)	0 (0)	6.87 (1.99)
3	5.00 (2.08)	3.33 (1.02)	0 (0)	7.32 (1.71)

of residual male olfactory cues encountered by a female before direct exposure to a male was associated with the emergence of a dramatic difference between rates of ultrasound emission observed during the pre- and post-male segments of testing. Thus, average rates of calling during the post-male periods of the first two estrous tests reliably exceeded those during the pre-male segment of the same tests ($p < .012$ for each of the first two tests, sign test).

Furthermore, it is clear that the frequency with which "estrous calls" are emitted by female hamsters prior to heterosexual contact increased during a series of tests on closely-spaced estrous days (Table X). Rates of ultrasound emission during the pre-male test segment were significantly greater during the third test than in either of the preceding two tests ($p \leq .046$ in each case). A similar difference is absent during any other segment of these tests. As a result of this significantly increased pre-male ultrasound rate during the third test, rates of calling in the pre- and post-male segments of this test are statistically indistinguishable. In view of the frequent and thorough rinsing of test cages and the appearance of a significant pre-post difference during each of two previous tests, it seems unlikely that the increased rate of pre-male calling, and the associated disappearance of a pre-post difference, during the third test stemmed solely from a build-up of male olfactory cues over successive tests.

Results summarized in Table X also indicate that high-frequency sounds are not produced by estrous females during maintenance of the rigid lordosis posture following the male's departure. We have inferred that "ultrasounds" emitted by pairs composed of an active male and an estrous female in lordosis are attributable to the male partner (Chapter V; also see Experiments 7-8 and 13-14 of this chapter).

Discussion

Results summarized in Table X indicate that when persistent olfactory and experiential cues are reduced to minimal levels, brief exposure to a male causes an immediate and sustained increase in the rate with which high-frequency sounds are emitted by estrous female hamsters. However, these results also suggest strongly that repeated exposure to a rigid experimental routine may facilitate learning or conditioning to the situation which, itself, suffices to "prime" a female prior to anticipated heterosexual contact. Both of these relationships are highly consistent with the existence of an adaptive "strategy" according to which any stimulus or cue associated with a higher-than-normal probability of finding a male in the immediate vicinity will provoke an increased rate of ultrasound emission on the part of an estrous female.

EXPERIMENT 10: THE STIMULATION OF FEMALE CALLING BY EXPOSURE TO MALE SHAVINGS

A clear implication of hypotheses such as that outlined above is that olfactory cues indicative of a male's proximity should provoke increased rates of "ultrasound" emission by females. Effects of olfactory cues on female calling have been tested in an experiment in which soiled shavings from a male's home cage served as a source of male odors.

Method

Subjects and Test Procedures

Each of 10 adult females participated in 9-10 tests including at least 2 exposures to shavings on each of the 4 days of the female estrous cycle. During each test, a female was observed for 3 minutes prior to the introduction of male shavings, for 30 seconds in the presence of a small tray containing newly collected shavings from random locations within the home cage of a single adult male, and for 3 minutes following removal of the tray and its remaining contents (some typically were scattered about the female's home cage during her investigation). Participants in this experiment also were exposed to an awake male in the course of Experiment 7. While the two test series ran concurrently, the order in which individuals were exposed to the two types of stimuli was counterbalanced for equal groups of 5 females. Individuals first experienced 9-10 tests under one stimulus condition, and only after a 4-6 week delay did they begin the second series of 9-10 tests with the other stimulus. Preliminary analyses indicated that results of these tests were independent of the order in which individuals experienced the two test series. Therefore, results have been combined according to stimulus condition and discussed as two separate experiments (see Experiment 7).

Results

Throughout the female estrous cycle, rates of high-frequency vocalization in the presence of male-shavings exceeded those before and after stimulus presentation (Table XI). However, statistically

Table XI: Mean Rates of Ultrasound Emission Before, During and After Exposure to Male Shavings.

Cycle Day	Mean Ultrasounds/Minute (SEM) Pre-Shavings	Male Shavings Present	Post-Shavings
1 (Estrous)	0.40 (0.16)	2.30 (0.71)	0.23 (0.12)
2	0.07 (0.05)	1.90 (1.58)	0.05 (0.03)
3	0.05 (0.03)	1.30 (0.89)	0.02 (0.02)
4	0.21 (0.11)	2.37 (1.89)	0.11 (0.07)

significant differences between "shavings-present" and other (pre- and post-shavings) test segments were observed only during tests on estrous day ($p \leq .02$, comparing average call rates for Day 1 females in the presence of shavings with those pre- and post-shavings, t-tests).

Rates of female calling during pre- and post-shavings segments of nonestrous tests tended to be considerably lower than those observed in the comparable segments of Day 1 tests (Table XI). In fact, at extreme points within the estrous cycle (Days 1 versus 3, see Table XI) rates of "ultrasound" emission pre-shavings differed significantly ($p = .048$, t-test), while differences in the post-shavings segments of the same tests approached statistical reliability ($t = .090$, t-test). Thus, female call rates prior to or immediately following presentation of male shavings tended to fluctuate with estrous state. In contrast, rates of "ultrasound" emission in the actual presence of male shavings tended to be uniformly high throughout the estrous cycle, exhibiting little or no estrous cyclicity (Table XI).

Finally, direct comparisons of pre-shavings call rates with post-shavings rates on the same cycle day (e.g., average Day 1 rates pre- versus post-shavings) failed to reveal any consistent pre-post differences at any stage of the female cycle (Days 1-4, see Table XI).

Discussion

Previous results (Experiments 7-8) have indicated clearly that female hamster "ultrasounds" encode information regarding reproductive state (as well as location). At least during test series with awake males, basal (pre-male) rates of vocalization by estrous females greatly exceed those typical of nonestrous females. Further, even higher rates of calling by estrous females may be provoked by transient exposure to an awake male (post-male periods in Experiments 8-9). Here, too, female call rates depend upon endocrine state, estrous females typically achieving higher post-male call rates than non-estrous females (Experiments 7-8). In the present experiment, we have demonstrated that estrous females (possibly nonestrous females as well, see Table XI and discussion below) also vocalize at higher rates during exposure to male shavings than either before stimulus presentation or after stimulus removal. These results indicate that male olfactory cues can stimulate the production of high-frequency sounds by female hamsters. This suggests that high rates of female calling seen in previous experiments also were attributable, in part, to stimulation by male odors. In particular, olfactory stimulation of female calling could have been involved in the high vocalization rates observed on estrous days in pre-male segments of Experiments 7-8 and post-male segments of Experiments 7-9.

High, and relatively constant, rates of female calling in the presence of male-shavings demand some explanation in terms of the hormonal basis for female vocalization. During informal observations, we have found that the combination of estradiol benzoate and progesterone is sufficient to support high rates of "ultrasound" emission by ovariectomized females in situations similar to the pre- and post-male segments of Experiments 7-9 (unpublished observations). It appears that one or both of these hormones underlies the marked cyclicity in call rate exhibited by intact females. However, the present

results indicate that female "ultrasound" rates in the presence of male shavings tend to be uniformly high during estrous and nonestrous phases of the female cycle, showing no pronounced tendency to fluctuate with hormonal state. Thus, these results suggest that any hormonal stimulation of calling may be modulated by external stimuli which themselves inform a female as to the probability of male-female contact. Cues normally associated with the physical presence or very close proximity of a male (e.g., shavings from a male's home cage) may elicit high rates of ultrasound emission without the high levels of hormonal "priming" required in less effective stimulus situations. Such interactions between hormonal and stimulus effects have been described previously for mating behaviors (Diakow, Pfaff & Komisaruk, 1973).

EXPERIMENT 11: THE STIMULATION OF FEMALE CALLING BY EXPOSURE TO ANESTHETIZED MALES

Brief contact with male shavings increases the rate at which estrous female hamsters emit high-frequency calls (Experiment 10). Thus, male odors can stimulate "ultrasound" production by estrous females. This suggests that high rates of female calling observed during tests with active males (see Experiments 7-9) are mediated, at least in part, by male olfactory cues. On the other hand, throughout the estrous cycle, rates of female "ultrasound" emission pre- and post-exposure to awake males (Table VII) exceeded those pre- and post-male shavings (Table XI). Such differences could imply that male shavings are a suboptimal source of male odors. In order to clarify the role of olfactory cues in the stimulation of female ultrasound production, it seemed advisable to investigate female responses to other sources of male odors. Therefore, in an attempt to take advantage of a strong and inclusive source of primarily olfactory cues, the present experiment has employed anesthetized males as sources of male odors (see also Experiment 12).

Method

Subjects and Test Procedures

Each of 10 naive females encountered an anesthetized male during two tests separated by a two-day interval. Half of the females were tested first on Day 1 (estrous day) of the cycle, and then on Day 3; the other half experienced tests in the reverse order. Six different intact males served as experimental stimuli on a rotating basis. All tests were staged in the individual female's home cage. Tests included five minutes of observation prior to the introduction of the male, five minutes in the presence of the anesthetized male, and a final five-minute observation period following the male's removal (see Table VI). Males were anesthetized with 0.35 cc/100 gm of Equithesin administered intraperitoneally at least five minutes before exposure to the female.

Results

In tests at both stages of the estrous cycle (Days 1 and 3), average rates of high-frequency sound emission by females were greater in the presence of an anesthetized male than before his introduction or following his removal (Table XII). This difference,

Table XII: Mean Rates of Ultrasound Emission Before, During and After Exposure to an Anesthetized Male.

	Mean Ultrasounds/Minute (SEM)		
Cycle Day	Pre-Male	Anesthetized Male Present	Post-Male
1 (Estrous)	0.18 (0.09)	1.18 (0.63)	0.36 (0.13)
3	0.06 (0.04)	1.27 (0.67)	0.02 (0.02)

however, is statistically significant only for comparisons of rates observed on Day 3 of the estrous cycle ($p = .032$ for each comparison, sign-test).

Rates of high-frequency calling provoked by the prior presentation of an anesthetized male (post-male period, Table XII) exhibited an estrous cyclicity similar to that seen in previous experiments with awake males (Experiments 7-8) and male-shavings (Experiment 10). Rates of calling during the post-male segment of Day 1 tests were approximately 18 times those seen near the midpoint of the nonestrous phase of the cycle (Day 3; $p = .031$, t-test). In contrast, rates of "ultrasound" emission in the actual presence of an anesthetized male (male-present segment in Table XII) did not show marked variations with the female estrous cycle.

Discussion

Anesthetized male hamsters are mute, and provide no tactile cues comparable to those associated with the close social investigation devoted to estrous females by active males. Such stimulus males provide visual cues, but ones quite different from those provided by normal active males. While anesthetized males may not differ markedly from active males in their susceptibility to gustatory investigation, females do not consistently take advantage of this by mouthing, biting or licking anesthetized stimulus males. In contrast, a test female presented with an anesthetized male invariably devotes considerable time to a thorough sniffing investigation of the stimulus animal, suggesting strongly that the most salient cues presented by inactive anesthetized males are male odors. Thus, the present results indicate that male odors can stimulate high-frequency calling by females. Maximal rates of ultrasound production by estrous and nonestrous females were achieved in the presence of anesthetized males. For nonestrous test females, these "male-present" call rates were significantly higher than those achieved before or after stimulus presentation. Higher and more variable basal call rates exhibited by estrous females may have accounted for their failure to consistently call at higher rates in the presence of anesthetized males. Nevertheless, this failure, together with the absence of a significant pre-male versus post-male difference in scores contributed by estrous or nonestrous females during tests with anesthetized males, suggests that anesthetized

males do not differ markedly from male shavings (Experiment 10) as sources of male odors (also compare average scores summarized in Tables XI and XII). Accordingly, we may question the exact degree to which male odors, as presented here, substitute completely for awake males (e.g., Experiment 9). However, in previous tests with awake males, the persistence of elevated call rates into the post-stimulus interval depended upon particular details of the testing regimes employed, specifically, the extent to which residual male odors were eliminated from observation cages between tests. The possible involvement of this factor in the results of tests with male shavings (Experiment 10) and anesthetized males (present experiment) has been examined in a subsequent experiment employing anesthetized males as odor sources (Experiment 12).

As in experiments with awake males, rates of calling by intact females following brief exposure to an anesthetized male depend upon endocrine state: Post-male rates on estrous day are significantly greater than those on nonestrous days of the female cycle. This is consistent with the interpretation of female high-frequency sounds as sexual attractants. On the other hand, female ultrasound rates in the presence of the anesthetized male on Days 1 and 3 of the female's cycle are similar. This, too, is similar to results of previous experiments with awake males. However, in contrast to these earlier experiments, rates of male-present calling here clearly are uncontaminated by signals produced by the male. Together with previous results describing the responses of females to another source of male odors (male-shavings, see Experiment 10), this finding suggests that responses to strong stimuli which ordinarily would be tied inextricably to the presence of a male may not be as susceptible to hormonal control as responses to less compelling stimuli.

EXPERIMENT 12: PRE- VERSUS POST-STIMULUS DIFFERENCES IN FEMALE CALLING TO AN ANESTHETIZED MALE

In Experiments 10 and 11, elevations of female vocalization rate in response to male olfactory cues occurred only in test segments during which the odor source was physically present (stimulus-present

test segments), and did not persist beyond the removal of the olfactory stimulus. Thus, experiments dealing with effects of male-shavings (Experiment 10) and anesthetized males (Experiment 11) did not show pre-post (pre-stimulus versus post-stimulus) differences in call rate comparable to those seen with presentations of awake males to estrous females (Experiment 9). However, with awake males as stimuli, pre-post differences in estrous female vocalization rates appeared only when test cages were rinsed thoroughly between successive tests, suggesting that male odors could persist in uncleaned cages and increase call rates during pre-male segments of subsequent tests, effectively eliminating any differences between pre- and post-male call rates (Experiment 9). Thus, it seemed possible that, in anesthetized-male presentations, too, pre-post differences in female ultrasound rates might have been masked by a persistence of male odors throughout a series of tests in the female's infrequently cleaned home cage (Experiment 11). This possibility was tested by again exposing females to anesthetized males, but in freshly cleaned test cages instead of the test female's home cage.

Method

Subjects and Test Procedures

Each of 11 cycling females experienced a series of two tests on closely-spaced estrous days. Each individual had received some previous experimental experience. For example, all had participated 4 weeks previously in Experiment 9. However, statistical analyses of data from this and other experiments have indicated that "rest periods" of several weeks were successful in eliminating spurious effects due to differences in degree of prior experience.

Tests were conducted in 48 x 27 x 20 cm polycarbonate cages. To minimize the persistence of male olfactory cues over successive tests, cages were rinsed thoroughly in hot tap water after each test. Soiled shavings were removed from test cages before washing, and later were replaced with clean cedar shavings.

Each test female was paired with a different intact adult male and the composition of pairs was held constant throughout testing. In fact, with a single exception, each experimental female retained the same male partner as in a previous experiment (Experiment 9) utilizing nearly identical test procedures, but comparing female vocalization rates before, during and after exposure to an awake male. In the present experiment, test females were exposed briefly to anesthetized males. Males were anesthetized with 0.35 cc/100 gm of Equithesin administered intraperitoneally at least 5 minutes before male-female contact.

As in the previous experiment with awake males (Experiment 9), tests here included: a 5 minute pre-male period during which the test female's "spontaneous" rate of vocalization was observed; a 60 second period during which each female was exposed to her anesthetized partner; and, a final 5 minute observation period following the gentle removal of the anesthetized male (also see Table VI). Each of two test females performed lordosis for 20-30 seconds following the removal of the stimulus male (no high-frequency calls were observed during either period). In these cases, as in previous experiments, the beginning of the post-male test segment was delayed until the female initiated locomotion.

Results

When persistent male odors were reduced to minimal levels, estrous females vocalized at significantly higher rates in the presence of an anesthetized male than they did preceding his introduction into the test cage ($p = .024$, t-test, see Table XIII). Further, elevated rates

Table XIII: Mean Rates of Female Calling Before, During and After Brief Exposure to an Anesthetized Male.

Mean Ultrasounds/Minute (SEM)		
Pre-Male	Anesthetized Male Present	Post-Male
1.14 (0.33)	4.18 (1.01)	4.13 (1.16)

of female calling persisted into the test segment following removal of the anesthetized male (post-male segment, see Table XIII). Here, too, rates of ultrasound emission reliably exceeded "spontaneous" rates prior to stimulus presentation ($p = .017$, t-test). The elevated rates of female vocalization achieved during male-present and post-male test segments were statistically indistinguishable. Similarly, no reliable differences within any test segment could be detected comparing the first and second tests experienced by each of the 11 females.

Discussion

Under appropriate testing conditions, brief exposure to an anesthetized male clearly can cause an immediate and sustained increase in the rate with which high-frequency sounds are produced by estrous female hamsters. These results suggest that: (a) male odors can facilitate female calling (also see Experiment 10); (b) the elevated rates of female vocalization occasioned by male odors persist for some time following termination of contact with the odor source; and, (c) high rates of female responding following (and possibly during) exposure to awake males stem, in part, from the olfactory cues provided by the awake males (also see Whitney et al., 1973 and 1974). All of these relations are highly consistent with the interpretation of high-frequency hamster calls as communications by which females indicate their locations and endocrine state to nearby males. In particular, these findings suggest that male "scent-marks," or simply male-odors incidental to the earlier presence of an active male, could normally increase the repetition rate of high-frequency calls by an estrous female encountering those cues. The dependence of female "ultrasound" emission on male olfactory cues suggests that these signals normally would be produced at high rates only when a male is near and might be attracted, thus facilitating intraspecific contact while minimizing any attraction of potential predators. Moreover, a higher rate of calls also could greatly facilitate the localization of the estrous female by the male odor source. In the course of any such interaction, the relatively precise and immediate spatial information encoded in acoustic signals

would complement more persistent olfactory cues deriving, for instance, from vaginal discharge odors (e.g., see Johnston, 1974d). Female acoustic signals depend upon endocrine state (Experiments 7-9), male olfactory cues (Experiments 10-12) and possibly other male signals (note the higher rates of female calling evoked by awake males in Experiments 7-9 as compared with those elicited by male odors in Experiments 10-12; also see Chapter VII). Since hamster glandular secretions and olfactory scent-marking behaviors also depend upon endocrine state (e.g., see Orsini, 1961; Johnston, 1970a and 1974d; Vandenbergh, 1971 and 1973; Drickamer & Vandenbergh, 1973; and, Drickamer, Vandenbergh & Colby, 1973), a very general implication of our results is that successful reproduction among hamsters is facilitated by a reciprocating chain of acoustic and olfactory communications in which each element signals the sexual receptivity (endocrine state), relative aggressiveness and location of its source (also see Chapter VII).

EXPERIMENT 13: VARIATIONS IN MALE VOCALIZATION RATE WITH EXPOSURE TO ESTROUS AND NONESTROUS FEMALES

High rates of calling by males may have accounted, in part, for the high vocalization rates observed during male-female pairings (Experiments 7-9). This notion was tested in observations of high-frequency vocalizations by male hamsters, including comparisons of male call rates before and after exposure to females of known estrous state.

Method

Subjects and Test Procedures

Each of 12 adult males was paired with a different intact female of known estrous cycle (see Orsini, 1961). Test males participated in a series of 4 tests within 8-10 days. Each of 6 males experienced his first test on the appropriate stimulus female's estrous day (Day 1). Each of the remaining 6 males was tested initially on his female's Day 3 (the midpoint of the nonestrous phase of the 4-day female cycle). Every male eventually accumulated two tests on his partner's estrous day, and two on her Day 3. All stimulus females maintained

regular 4-day estrous cycles throughout testing. Each test male had received a very limited extent of previous experimental experience. However, differences in degree of previous experience seemed unrelated to any of the experimental results to be summarized here.

During each test, males were monitored for the production of high-frequency sounds for 5 minutes prior to the introduction of any stimulus (see Table VI). This "pre-female" period was followed immediately by the introduction of the test individual's stimulus female. The rate of ultrasound emission then was noted during a 60-second period of free male-female interaction. If lordosis was performed by the stimulus female, its latency was recorded, and high-frequency calls were tabulated separately for periods during active locomotion by the stimulus female and during lordosis (once initiated, lordosis was invariably maintained throughout the remainder of this 1 minute period). Finally, test males were observed for an additional 5 minute post-female period following the removal of the stimulus female. Test cages then were rinsed thoroughly in hot water, dried and filled to a depth of roughly 1-2 cm with clean cedar shavings prior to the subsequent test.

Results

Adult male hamsters emitted high-frequency calls at low basal (pre-female) rates of 0.2 - 0.7 calls/minute (Table XIV). The physical presence of an awake estrous or nonestrous female (female-present test segments, see Table XIV) was associated with much higher rates of high-frequency sound emission than observed pre-female ($p \leq .012$, sign test). However, female-present rates on Days 1 and 3 of the stimulus female's estrous cycle differed significantly from each other ($p = .022$, t-test), rates in the presence of a Day 3 female exceeding those associated with pairings of males with Day 1 females. Since lordosis was a highly prepotent response for Day 1 females (mean lordosis latency = 8.4 sec., standard error = 1.5 sec.), but never was performed by Day 3 females, and since females do little or no calling during lordosis (Experiment 3), a female's contribution to overall call rates during female-present segments of Day 1 tests could have been

Table XIV: Male Vocalization Rates Before, During and After Exposure to An Awake Female

	Mean Ultrasounds/Minute (SEM)					
		Female Present Rates			Post-Female Rates	
Cycle Day of Stimulus Female	Pre-Female, Overall Rate	Overall	Pre-Lordosis[a]	Lordosis[b]	Overall	1st Minute
1 (Estrous)	0.67 (0.59)	3.79 (0.63)	17.46 (2.81)	2.14 (0.62)	2.01 (0.69)	5.29 (1.11)
3 (Nonestrous)	0.17 (0.08)	10.17 (2.49)	[c]	[c]	0.36 (0.15)	1.63 (0.72)

[a] Prior to the exhibition of lordosis by the stimulus female.

[b] During the performance of lordosis by the stimulus female. Once initiated, lordosis was maintained throughout the remainder of the female-present test segment.

[c] No lordosis occurred during nonestrous tests.

much lower than during Day 3 tests. Such differences in female contribution would probably be sufficient to account for the difference in female-present call rates on Days 1 and 3 of the stimulus female's cycle (Table XIV).

Average male vocalization rates following brief exposure to an estrous female consistently exceeded corresponding pre-female rates ($p = .038$, sign test, see Table XIV). However, pre- and post-female rates during Day 3 tests did not exhibit such reliable differences ($p = .124$, t-test). Accordingly, direct comparisons of male call rates following exposure to Day 1 versus Day 3 females revealed consistent differences. Previous exposure to an estrous female elicited significantly higher rates of male vocalization (post-female rates, see Table XIV) than did exposure to a Day 3 female ($p = .012$, sign test).

Male call rates also tended to vary <u>within</u> the 5-minute post-female test segment, decreasing from initially high rates during the first minute of post-female observation to significantly lower rates during the remainder of this test segment. For example, average rates during the first minute of post-female segments (5.3/minute and 1.6/minute on Days 1 and 3, respectively) were significantly higher than overall rates during the corresponding post-female test segments ($p < .006$, sign test, see Table XIV). Similarly, average rates during the first minute post-female consistently exceeded those during the fifth minute of the same test segments (0.25 and 0.04 calls/minute during the fifth minute post-female on Days 1 and 3, respectively; $p \lesssim .054$, t-test). As in the case of overall averages, mean vocalization rates in the first minute post-female of estrous-day tests consistently exceeded rates during the same segment of nonestrous tests ($p = .006$, sign test). In contrast, average rates during the fifth minute post-female did not exhibit significant variations with estrous state.

Discussion

These results prove that male hamsters do emit high-frequency vocalizations (see also Sales, 1972a and b). In fact, pre- and post-stimulus call rates by males fall well within the ranges established by estrous and nonestrous females exposed to similar experimental paradigms (Experiments 7-9). Direct observations of male calling confirm earlier inferences based on calling by male-female pairs during the performance of lordosis by the female partner (Experiment 9; see also Chapter V).

Male call rates depend upon the estrous state of the stimulus females. A significant elevation of post-female call rates over pre-female rates was seen only in association with presentations of estrous females. As a consequence, male ultrasound rates following brief exposure to an estrous female consistently exceeded those provoked by brief contact with a nonestrous (Day 3) female. The ability of awake females to stimulate male calling, and the dependence of male call rates on female reproductive state, suggest that male ultrasounds, like their female counterparts, operate under natural conditions to facilitate male-female contact leading to successful reproduction.

Male ultrasound rates consistently decrease in the course of a 5-minute test following exposure to a stimulus female. Rates of male calling also may fluctuate within female-present segments of Day 1 tests, depending on whether the stimulus female is active or has adopted the rigid lordosis posture. For example, average call rates during the performance of lordosis by an estrous stimulus female were much lower than rates prior to the female's adoption of lordosis (2.1/minute during the 52 seconds of maintained lordosis versus 17.5/minute during the 8 seconds preceding adoption of lordosis, $p = .006$, sign test). Finally, male vocalization rates in the presence of a female in lordosis are consistently lower than those observed immediately following the female's removal, (2.1/minute during lordosis versus 5.3/minute during the first minute of the post-female period, $p = .006$, t-test). Together, these observations suggest that male

call rates may depend upon the "task at hand." Very high rates may be required for (a) the induction or facilitation of lordosis in an active female (or the inhibition of her aggression), and, (b) facilitation of the prompt resumption of contact with an estrous female immediately following accidental separation. In contrast to these situations, the maintenance of lordosis in an estrous female already in this posture may constitute a much less demanding task requiring fewer male ultrasounds.

EXPERIMENT 14: MALE VOCALIZATION RATES BEFORE, DURING AND AFTER EXPOSURE TO ANESTHETIZED FEMALES

Brief exposures to awake females can provoke increased rates of high-frequency calling by male hamsters. However, significantly elevated post-female call rates were seen only following presentations of estrous (Day 1) females (Experiment 13). The ability of males to discriminate between estrous and nonestrous females could have been based on differences in female behavior (e.g., the performance of lordosis by estrous females or the exhibition of intense aggression by nonestrous females; see Chapter III), on female olfactory or acoustic cues, or on some other signal. In particular, female odors influence other aspects of male hamster behavior (e.g., Johnston, 1970a), suggesting that they could account wholly or in part for the stimulation of male vocalization (also see Whitney et al., 1973 and 1974). Further, an odorous vaginal discharge varies in consistency and quantity during the 4-day female cycle, appearing in larger amounts during estrous than on Day 3, near the midpoint of the nonestrous phase of the cycle (see Orsini, 1961). This secretion is attractive to males (Johnston, 1970a and 1974d; Murphy, 1973; and, Gregory et al., 1974) and could underlie the differences in male vocalization rate associated with presentations of Day 1 and Day 3 awake females (Experiment 13). The dependence of male ultrasound rates on female odors was investigated in an experiment in which males were presented with anesthetized females and observed before, during and after stimulus presentations for rates of high-frequency sound emission.

Method

Subjects and Test Procedures

Each of 12 adult male hamsters participated in a series of 4 tests within a period of 8-10 days. Each male was paired with the same female partner throughout testing (a different female for each of the twelve males), and the total of 4 tests were divided equally between the stimulus female's estrous day (Day 1) and a day near the midpoint of her nonestrous phase (Day 3). Test series were counterbalanced with respect to the female cycle day on which different male-female pairs initially were tested. Male-female pairings observed during the present experiment were identical to those participating in a previous experiment investigating effects of exposure to awake females on male vocalization rates (Experiment 13). Test schedules here also were very similar to those employed previously. However, the initial tests of the present series were conducted at least 5 weeks after the last tests included in Experiment 13, an interval previously found adequate to minimize effects due to prior experimental experience (also compare spontaneous (pre-female) call rates in Tables XIV and XV).

During each test, males were monitored for the emission of high-frequency sounds for 5 minutes prior to the introduction of the stimulus female (see Table VI). This "pre-female" period was followed immediately by the introduction of the anesthetized stimulus female. Following a 60-second "anesthetized-female present" period, test males were observed for a final 5-minute period following the female's removal. As usual, test cages were thoroughly cleaned between successive tests to minimize any accumulation of persistent female odors.

Stimulus females were anesthetized with an intraperitoneal injection of 0.35 cc per 100 gm body weight of Equithesin at least 5 minutes prior to their participation in each test. Throughout the testing period, special attention was devoted to the degree to which stimulus females maintained regular estrous cycles despite frequent anesthetization. Cycles were monitored regularly (Orsini, 1961) and no female varied from the usual 4-day pattern at any point during testing.

Results

Male hamsters exhibited higher rates of ultrasonic vocalization in the presence of an anesthetized female than either before or after her presentation ($p \leq .038$ for all comparisons, sign test or t-test, see Table XV). This effect did not depend upon the estrous state of the stimulus female. Similarly, post-female call rates consistently exceeded pre-female rates regardless of the cycle stage of the anesthetized stimulus female ($p \leq .066$, sign test, see Table XV).

No significant difference in average male call rates was detected over successive tests on the same cycle day (2 tests on each of cycle days 1 and 3). As in Experiment 13, however, males did tend to decrease in call rate in the course of the post-female observation period. Males vocalized at significantly higher rates during the first minute following exposure to Day 1 or Day 3 females than they did throughout the remainder of the post-female period (Table XV). Hence, call rates during the first minute post-female consistently exceeded average rates for the entire test segment, including the first minute ($p \leq .012$, sign test).

Discussion

Male hamsters call at low basal rates, but respond to brief contact with anesthetized females with significantly increased ultrasound rates. The ability of anesthetized females to stimulate male calling suggests that female olfactory cues are important determinants of male vocalization rates (see also Whitney et al., 1973 and 1974). Anesthetized females are mute, and any visual or tactile cues they provide must differ greatly from those normally provided by awake estrous or nonestrous females. On the other hand, gustatory cues provided by anesthetized females may not differ significantly from those offered by awake females. However, within the one minute periods of free access to stimulus females, males did not consistently mouth, bite or lick their anesthetized partners. In contrast, olfactory investigation (sniffing) constituted a highly prepotent response for males confronted with anesthetized females. Thus, we conclude that female odors emanating from the anesthetized

Table XV: Male Vocalization Rates Before, During and After Exposure to an Anesthetized Female

	Mean Ultrasounds/Minute (SEM)			
			Post-Female Rates	
Cycle Day of Stimulus Female	Pre-Female, Overall Rate	Female Present, Overall Rate	Overall	1st Minute
1 (Estrous)	0.25 (0.13)	3.28 (0.77)	0.96 (0.31)	2.82 (0.86)
3 (Nonestrous)	0.26 (0.11)	4.10 (0.93)	0.59 (0.16)	2.15 (0.67)

stimulus females employed here accounted for most or all of the increases in rates of male calling and that future experiments utilizing more pure sources of female odors will be marked by similar effects on male rates of ultrasound emission.

While female odors seem to be the most salient cues provided by anesthetized females, other types of cues probably contribute to rates of male calling provoked by awake females. In fact, rates of male calling in the presence of anesthetized females do not closely approach the very high ultrasound rates emitted by pairs composed of males and awake females (Experiment 13). Further, female rates of response to anesthetized males (Experiments 11 and 12) are very similar to those exhibited by males in the presence of anesthetized females. In each case, ultrasound rates achieved by the one active pair member are insufficient to account for the total rate when both are active. In most cases, even the sum of odor-stimulated call rates by males and females is considerably less than rates achieved during active heterosexual interaction. Thus, male-female social interactions involving a variety of sensory modalities (e.g., see Chapter VIII) seem likely to be important factors determining net vocalization rates by active pairs.

In terms of vocalization patterns, males do not seem to discriminate reliably among anesthetized females of different estrous conditions, suggesting that males may not respond differentially to olfactory cues provided by Day 1 and Day 3 females, but may discriminate among such females only on the basis of their gross behavioral reactions (sexual receptivity and acceptance by estrous females; resistance and aggression in the case of Day 3 females). This interpretation is consistent with data (see Johnston, 1974d) suggesting that female hamster vaginal secretion collected during estrous and nonestrous phases of the reproductive cycle does not differ in attractiveness to males. However, males do tend to scent-mark at very different rates in the home cages of estrous as opposed to nonestrous females, a discrimination presumably based upon olfactory cues (Johnston, 1970a). These results suggest that the method used to present female odors could affect the male's ability to discriminate between estrous and nonestrous females.

For example, the sensitivity of male hamsters to these odors may be sufficiently great that males respond maximally to very small quantities (such as might actually be deposited during scent-marking within a home cage), and larger quantities (such as might be presented during preference tests (see Johnston, 1974d) or by an anesthetized female) consequently may fail to elicit any more pronounced response regardless of the estrous state of the odor source. Such a relationship would seem well suited to hamster social organization. Given the extreme and indiscriminate aggressivity of nonestrous female hamsters (e.g., Chapter III), total failure on the part of males to respond differentially to estrous and nonestrous females could be highly maladaptive. In fact, reproductive success might be best served by discriminations based on forms of distance communications, such as olfactory scent marks or high-frequency acoustic signals. This would have the advantage of allowing discrimination to occur at relatively preliminary (and safe) links in a long chain of events leading to the achievement of close proximity and the possibility of direct social interaction. Discrimination might be essentially complete before male and female make physical contact, and might actually be less critical during subsequent phases of their interaction despite the presence of potentially stronger stimuli.

GENERAL DISCUSSION

Our investigations of the stimulus determinants of high-frequency vocalizations by male and female hamsters have produced data relevant to the functional interpretations of male and female ultrasounds. On the female side, all of the findings summarized above are consistent with a view of female hamster ultrasounds as sexual advertisements which indicate the locations, receptivity and relative passivity of estrous females to nearby male conspecifics. Since estrous females do not call during lordosis, female ultrasounds probably are infrequent <u>during</u> heterosexual contact and may operate primarily as noncontact signals, attracting males to an initial rendezvous, or facilitating the resumption of contact following accidental separation. The dependence of female

ultrasound emission on olfactory cues suggests that these signals would be produced most frequently when a male is within earshot and might be attracted, thus facilitating intraspecific contact while minimizing the chances of attracting potential predators.

Rates of ultrasound emission by male hamsters also depend upon heterosexual contact, call rates typically increasing with brief exposure to awake or anesthetized females. Further, male ultrasound rates following contact with awake females depend upon the estrous state of the stimulus female, post-female rates of calling elicited by Day 1 (estrous) females consistently exceeding those provoked by Day 3 (nonestrous) females. Thus, high-frequency calls by male hamsters seem likely to function normally as sexual signals, operating to facilitate male-female contact leading to reproduction. On a very general level, then, we have arrived at similar interpretations of the functions of male and female ultrasounds. Our next step might be to ask if male and female ultrasounds are functionally identical, or whether they differ in some aspect of the message that each conveys to an opposite-sex conspecific. In fact, several forms of very indirect evidence do suggest slight differences in the functions of male and female ultrasounds. In particular, males seem to emit high-frequency calls at somewhat lower rates than females exposed to similar experimental paradigms. This difference may be especially pronounced in situations in which the test subject (male or female) is observed in the absence of a conspecific (pre-stimulus or post-stimulus; e.g., compare Table XIV with Tables VII, VIII and X). Further, male rates of calling seem to decrease more rapidly following the removal of an effective stimulus than do female rates in similar situations. For example, relative to average call rates during the first minute of a 5-minute post-stimulus test segment, males (Experiment 13) exhibited significantly lower rates during the final minute of testing than did females (Experiment 9; $p < .001$, t-test for independent means). Both of these observations suggest that male calls may be more closely tied to the immediate physical presence of a female than are female calls to the presence of a male. In turn, this suggests that male calls may function to facilitate reproduction

primarily during heterosexual contact, possibly by reducing the latency to the performance of lordosis by a receptive female, by prolonging lordosis following its initial appearance, or both (see Chapter VII). These speculations also are consistent with differences in the physical characteristics of male and female ultrasounds (Chapter V). Female ultrasounds tend to include some rapid fluctuations in amplitude or frequency, physical characteristics which would facilitate the localization of a female sound source over moderate distances. In contrast, male calls tend to be more constant in volume and frequency, and, thus, would be more difficult than female calls to localize over any distance. Male calls might, then, be most effective at closer range, following the achievement of initial social contact. Such a role might neatly complement the communicatory value of female calls and of other modes of hamster social communication, thus adding to an extensive system of social signals which help to coordinate endocrine (reproductive) state with appropriate social behaviors.

CHAPTER VII. RESPONSES OF HAMSTERS TO PLAYBACKS OF NATURAL AND SYNTHETIC ULTRASOUNDS

Golden hamsters of both sexes emit high-frequency sounds ("ultrasounds") during male-female or female-female pairings (Sewell, 1970; Sales, 1972a; Chapters V-VI). Female calls convey information regarding reproductive state, and may function primarily as sexual attractants. High-frequency sounds are emitted most often by an isolated female when she is most sexually receptive (Sales, 1972a; Chapter VI). However, even higher rates of calling by estrous female may be provoked by cues associated with the presence or proximity of a male. For example, an awake male, an anesthetized male, or shavings from the home cage of a male, are adequate to facilitate female calling (Chapter VI). In each case, olfactory cues provided by a male probably are instrumental in provoking the observed increase in vocalization rate. These findings all are consistent with a view of female hamster ultrasounds as noncontact sexual advertisements which indicate the locations, receptivity and relative passivity of estrous females to nearby male conspecifics. Physical characteristics and stimulus determinants of ultrasounds produced by isolated male hamsters suggest that they, too, could function as sexual attractants over short to moderate distances (Chapters V-VI; Floody & Pfaff, 1974). However, male calls emitted in the presence of an estrous female seem less suited to distance communication and may play some other role in hamster social behavior and communication.

Ultimately, functional interpretations of rodent vocalizations should be based on consideration of the behavioral consequences, as well as the physical properties and stimulus elicitors, of these sounds (e.g., Bell, 1974). High-frequency acoustic signals have been elicited from infant and adult representatives of nearly all rodent species examined (e.g., Anderson, 1954; Zippelius & Schleidt, 1956; Noirot, 1970 and 1972; Allin & Banks, 1972; Barfield & Geyer, 1972; Sales, 1972a and b; Brooks & Banks, 1973). For example, isolated infants emit ultrasounds when cold, and when subjected to rough tactile stimulation (e.g., Okon, 1972; Smith, 1972). Differences in the physical characteristics of calls emitted in these two types of

situations have suggested that infant vocalizations may convey two distinct messages, each corresponding to a distinct form of maternal response (Smith, 1972; see also, Noirot, 1972; and, Okon, 1972). In particular, cold-elicited calls have been associated with maternal attraction and retrieval, while tactually-provoked calls have been linked to immediate cessation of infant-maternal contact. However, direct tests of inferences such as these are rare and have focused on only a few of the responses reputedly elicited by infant ultrasounds. For example, playbacks of tape-recorded ultrasounds emitted by infant rats attract the attention of both lactating female rats and adult males, and can induce a lactating female to leave her nest, engage in searching behavior, and accurately localize the sound source (Allin & Banks, 1972). In similar experiments, Sewell (see Noirot, 1972) has compared the ability of various sounds to elicit approach by lactating female wood-mice (Apodemus sylvaticus). "Isolation calls" recorded from 5-day old infants usually elicited rapid and accurate approach by lactating females. In contrast, "foreign signals," including artificially generated 5-9 msec. pulses of 45 kHz, seldom elicited any response (also see Bell, Nitschke, Bell & Zachman, 1974; and, Smotherman, Bell, Starzec & Elias, 1974).

While adult responses to infant ultrasounds have been subjected to some direct investigation, interpretations of the communicative value of high-frequency sounds of adult origin have rested largely on temporal correlations between sound emission and other behaviors of the source and any recipients (e.g., Barfield & Geyer, 1972). We have attempted more direct tests of the functional significance of high-frequency signals emitted by adult male and female golden hamsters. We have found that playbacks of natural calls and of synthetic high-frequency sounds which resemble natural calls share the ability to elicit several well-defined and socially relevant behavioral responses. In particular, playbacks of synthetic ultrasounds stimulate high rates of calling by estrous females. Further, in a simple choice situation, adult hamsters of both sexes are able to localize a source of natural or synthetic ultrasounds, and are attracted to it. Finally, high-frequency sounds may induce lordosis in receptive females and prolong the maintenance of lordosis by receptive females following brief heterosexual contact.

GENERAL METHOD

Subjects and Maintenance

Random-bred male and female hamsters, of the LVG:LAK strain, were purchased from Lakeview Hamster Colony, Newfield, New Jersey. Individuals were 28-63 days old when acquired and each was housed separately in a metal cage of dimensions 25 x 18 x 18 cm or 30 x 30 x 18 cm. Hamsters were maintained as described in previous chapters. During testing, all participants were intact adults, ranging between 135 and 385 days of age. All experimental females maintained regular 4-day estrous cycles throughout testing (see Orsini, 1961).

Many individuals participated in several different experiments. Periods of several weeks generally intervened between successive experiments and individuals never participated in more than one concurrently. Nevertheless, most experiments to be described here included individuals of various ages and degrees of previous experimental or social experience. However, the composition of experimental groups was roughly balanced for each of these factors. Further, most groups participating in these experiments included some naive individuals, having received no experimental or social experience since their acquisition at least 3 months prior to the initiation of testing. Degree or character of previous experience never appeared to exert a consistent influence on experimental results. Therefore, results in individual experiments have been grouped without reference to previous experimental or social experience.

Instrumentation: The Detection, Generation and Reproduction of High-Frequency Sounds

Heterodyne ultrasonic detectors manufactured by Holgates of Totton, Southhampton, England (Holgate Ultrasonic Receiver, Mk. V) were used routinely for the simple detection of high-frequency hamster vocalizations. This sort of instrument translates high-frequency "ultrasounds" into audible sounds that may be counted directly by an observer.

Holgate receivers are relatively narrow-band instruments, their sensitivity typically decreasing with increasing differences between the frequency of an input signal (e.g., a natural vocalization) and the frequency to which the detector is "tuned." Our Holgate detectors were tuned to a central (most sensitive) frequency of 35 kHz, closely matching the frequency band most consistently emphasized in high-frequency vocalizations by male and female hamsters (Chapter V). Furthermore, variations in Holgate sensitivity with frequency were determined and compared with the frequency range and average intensity characteristic of hamster calls (Chapter VI). The results of these comparisons showed that the instruments and procedures employed here for the simple detection of hamster "ultrasounds" are appropriate to the physical characteristics of those signals and probably do detect virtually all calls emitted in situations such as those described in subsequent experiments.

The present series of experiments examines behavioral responses of hamsters to playbacks of tape-recorded natural hamster sounds, or "synthetic" (artificial) signals resembling natural calls. Natural hamster ultrasounds, calls emitted by a single estrous female shortly after brief exposure to a male, were tape-recorded at 30 inches per second on a Precision Instruments Co. tape recorder (model PS202). A wide-band ultrasonic receiver designed by the Lincoln Laboratory of Massachusetts Institute of Technology (see McCue & Bertolini, 1964) served as a transducer during the tape-recording of high-frequency sounds. A frequency band-width of recorded signals of approximately 15-50 kHz was imposed by filtering during the recording process (a Krohn-Hite filter, model 3500 R, operating with a pass-band of 15-100 kHz) together with the limited frequency response of the tape-recorder itself (at 30 ips, the approximate effective frequency range was 300 Hz - 50 kHz).

The fidelity (accuracy of frequency and waveform reproduction) of this entire recording system was checked periodically for known input signals consisting of 35 kHz sine waves. The tape-recorder output was monitored visually on an oscilloscope, and the accuracy of frequency

reproduction was confirmed at a tape speed of 30 ips for a wide range of input voltages. The fidelity with which fine details of the input waveform were reproduced depended on the amplitude of the input signal. For input signals of approximately 1 volt, a level used here in the generation of all tapes of synthetic ultrasounds (see below), the waveform of the output signal was indistinguishable from that of the original. For higher input voltages, such as the approximately 2-6 volt signals delivered to the tape-recorder during recordings of natural hamster sounds, the frequency of the input signal was preserved, but minor alterations in waveform were evident when compared to the original sinusoid.

For playbacks of natural hamster calls, a particular 30-second segment of the stimulus tape was selected on the basis of a relatively high and constant frequency of calls (an average of 3.5 per five seconds throughout its 30-sec. duration). In addition to the tape-recorder, play-back experiments required the following elements: a General Radio power amplifier, model 1233-A; a Hewlett-Packard attenuator set, model 350A; and, several two- or four-inch Kuhl-type ultrasonic transmitters (see Kuhl et al., 1954). The absolute intensities of all signals employed in playback experiments were determined with reference to a calibrated Bruel and Kjaer system including a B & K condenser microphone, type 4135, used in conjunction with a B & K cathode follower, type 2615, and microphone amplifier, type 2604.

Synthetic signals of 35 kHz and 100 msec. duration, with repetition rates of 1 or 0.1 per second were used as stimuli in some playback experiments. A previous chapter has described a configuration of equipment capable of generating constant tones of 35 kHz. Additional programming equipment (a Grason-Stadler electronic switch, model 829C, operated by an American Electronics Laboratories laboratory stimulator, model 104A) could be inserted into this system, providing complete control over the duration and repetition rate, as well as the frequency, amplitude and waveform, of our "synthetic" signals. Once generated in this manner, artificial hamster calls were recorded and reproduced in the same fashion as were natural hamster vocalizations (see above).

Statistics

The reliability of quantitative differences has been determined with the binomial test (Siegel, 1956) and with the t-test for dependent means, the t-test for independent means, and the t-test for single samples (Edwards, 1966). All probability values derive from two-tailed tests of significance.

EXPERIMENT 15: THE FACILITATION OF FEMALE CALLING BY TAPED ULTRASOUNDS

Male olfactory cues are associated with female vocalization rates that consistently exceed the low spontaneous rates characteristic of unstimulated females (Chapter VI). Nevertheless, call rates in the presence of male odor sources (in the absence of the male) do not closely approach total rates achieved during actual male-female social interactions. Since males, too, emit high-frequency sounds (e.g., Chapter V), the absence of an active calling male must account for part of the difference, simply by subtracting male calls from the total number generated by a male-female pair. However, the fact that rates of female calling post-male are considerably greater than rates following contact with separate male odor sources (Chapter VI) suggests that other factors also are involved. Thus, we considered the possible role of male vocalizations in the social interactions of active heterosexual pairs. It seemed possible that male calls might have direct facilitatory effects on female call rates.

Method

Subjects and Test Procedures

Following 15 minutes of pre-exposure to the 41 x 20 x 31 cm plexiglas test arena, each of ten estrous females was observed for the emission of high-frequency sounds for 5 minutes prior to the presentation of any stimulus (Pre-playback period, see Table XVI). Individuals then were exposed to playbacks of synthetic ultrasounds; in particular, 35 kHz sinusoids in pulses of 100 msec. duration, repeated at a rate of 1 per 10 seconds for 5 minutes. Finally, a 5-minute post-playback test segment immediately followed termination of the stimulus tape.

Table XVI: Mean Rates of Ultrasound Emission Before, During and After Exposure to Playbacks of Taped Ultrasounds.

Mean Ultrasounds/Minute, Estrous Day (SEM)		
Pre-Playback	During Playback	Post-Playback
0.52 (0.22)	8.24 (1.88)	1.80 (0.69)

With regard to frequency and waveform, synthetic pulses employed here as test stimuli resemble at least some natural hamster vocalizations, especially those emitted by males during contact with an estrous female (Chapter V). Further, the intensity of imitation calls also approximated natural levels. Thus, a female in an upright investigatory posture near the center of the test arena would be exposed to synthetic ultrasounds at an intensity of approximately 55 dB SPL (re. 2×10^{-4} dyne/cm^2). The choice of synthetic, rather than natural, high-frequency sounds stemmed from the fact that clear differences in intensity and temporal patterning allowed synthetic pulses to be distinguished easily from natural ultrasounds emitted by estrous test females. Thus, call rates summarized in Table XVI unequivocably include only female vocalizations in response to artificial stimulus calls; no stimulus calls were included among calls tabulated during playbacks of taped ultrasounds.

Results

Exposure to synthetic ultrasounds with physical characteristics similar to natural hamster calls resulted in a dramatic increase in the rate of emission of high-frequency sounds by estrous females (Table XVI). The average rate of vocalization during playbacks was consistently greater ($p \leq .004$, sign test) than rates observed before and after playbacks.

During experimental playbacks, natural calls tended to be concentrated early in the 10 second interval separating adjacent stimulus pulses, giving the impression that most female calls were emitted as immediate "answers" to stimulus calls. However, playbacks of synthetic ultrasounds seemed to have persistent, as well as immediate, effects on female hamster vocal behavior. Thus, facilitation of ultrasound emission by acoustic cues tended to persist beyond the termination of stimulus calls, into the post-playback test segment ($p = .076$, t-test, see Table XVI).

Discussion

It seems clear that acoustic cues such as might be provided by a male conspecific provoke high rates of ultrasound emission on the part of intact estrous female hamsters. To the extent that the calls so elicited might aid in the localization of the estrous female, these ultrasonic signals might facilitate sexual attraction. Repeated bursts of calls would be considerably easier to localize than infrequent calls emitted at rates as low as those exhibited by unstimulated estrous females. Conversely, the restriction of high vocalization rates to situations in which "advance notice" has been provided as to the proximity of a potential mate has obvious survival value in avoiding detection and localization by potential predators.

EXPERIMENT 16: TESTS OF THE ABILITY OF NATURAL AND SYNTHETIC "ULTRASOUNDS" TO ELICIT APPROACH IN A Y-MAZE

Several forms of indirect evidence (see Chapters V-VI; also Experiment 15 of the present chapter) have converged upon the interpretation that hamster ultrasounds function, at least in part, as sexual attractants (also see Sales, 1972a). To test this notion, the ability of high-frequency vocalizations to attract, or elicit approach on the part of, conspecifics must be examined. Thus, in several playback experiments, we have studied hamsters' ability to localize, and propensity to choose, a source of high-frequency sounds in a Y-maze (see Allin & Banks, 1972; and, Smotherman et al., 1974).

Method

Subjects, Materials and Test Procedures

Y-maze experiments were designed to determine if natural estrous female vocalizations and synthetic mimics of natural hamster calls are attractive to hamsters. Test subjects fell into several categories with respect to sex and endocrine state. Each of 5 males, 8 estrous (Day 1) females and 8 nonestrous (Day 3) females was exposed to natural ultrasounds in the Y-maze. Each of 10 males, 5 estrous females and 5 nonestrous females was exposed to synthetic high-frequency sounds. Each of four individuals (3 males and 1 female) exposed to synthetic ultrasounds previously had been exposed to natural ultrasounds in the Y-maze. However, the performance of these individuals did not change consistently with experience and was similar throughout both test series to the performance typical of totally naive subjects. Therefore, we have employed statistical tests for independent means in evaluating suggestive differences in average responses elicited by natural as opposed to synthetic ultrasounds.

The Y-maze employed in this series of observations was constructed of opaque plexiglas with a 9 x 9 x 34 cm long stem and two 9 x 9 x 51 cm long arms. To reduce potentially confusing internal reflections, most of the maze was lined with acoustic foam. For the same reason, a simple plexiglas baffle (a 9 x 9 cm piece of opaque plexiglas with a circular hole sufficient to allow the passage of a hamster) was permanently inserted into the stem of the maze near the choice point (the junction of arms and stem). Stimulus intensity determinations (see below) were conducted for each type of stimulus (natural and synthetic ultrasounds) and in each arm of the maze. These measurements were made within the maze arms, at points 35 cm from the Kuhl-type speakers located at the "goal" ends of the maze arms, and approximately 16 cm from the geometric center of the maze's choice area. Spatial restrictions prevented measurements of stimulus intensity within the choice area itself. However, sounds tend not to attenuate rapidly within highly confining channels such as the arms of the Y-maze, and it is unlikely that sound intensities at the choice point differed significantly

from those at slightly distal locations. A detailed description of procedures employed in determining average sound pressure levels is available in Chapter V.

Procedures involved in the recording and reproduction of stimulus tapes have been described (General Method; see also Chapter V). The stimulus tape of natural high-frequency vocalizations included calls by a particular female recorded on her estrous day. On the particular 30-second tape segment presented during each trial, calls appeared at an average rate of 3.5 calls per 5 seconds. The range of intensities for this selection of natural calls was approximately 48-50 dB SPL. The stimulus tape used in presentations of synthetic ultrasounds included 35 kHz sinusoids, in 100 msec. pulses repeated at a regular rate of 1 per second. The peak-to-peak intensity of each pulse was approximately 50 dB SPL. These levels of sound intensity approximate those at which hamsters normally emit high-frequency sounds (see Chapter V).

Each of the 37 hamsters participating in this series of observations experienced one or more experimental tests, each of which included a total of 11 trials in the Y-maze. The first trial was preceded by at least 20 minutes of free exploration of the maze. Thereafter, two-minute intervals separated successive trials. The location of the ultrasound source was varied randomly between arms within each series of trials. On each trial, the maze-arm chosen and the time from the beginning of the trial until the subject first approached within 15 cm of either speaker (latency to approach) were recorded. At the end of each 11-trial block, the parts of the maze not totally lined with acoustic foam were washed with paper towels soaked in warm water.

Results

Males, estrous (Day 1) females and nonestrous (Day 3) females all chose the sources of natural and synthetic ultrasounds more frequently than would have been expected on a chance basis ($p \leq .042$, see Table XVII). Estrous females tended to choose sources of natural, but not synthetic, ultrasound more reliably than did Day 3 females

Table XVII: Consistency of Approach to a Source of Ultrasounds in a Y-Maze.

	Mean Percent Choices of Ultrasound (SEM)	
	Natural Ultrasound	Synthetic Ultrasound
Male	74.5 (8.4)	62.7 (3.2)
Estrous Female (Day 1)	87.5 (5.2)	74.5 (7.8)
Nonestrous Female (Day 3)	72.7 (6.2)	63.6 (4.1)

($p = .088$, t-test for independent means). The percentage of choices of ultrasound exhibited by males was closer to the performance of nonestrous females, but was statistically indistinguishable from that of either female group regardless of ultrasound source.

The relative latencies exhibited by these various groups were consistent with trends outlined above (Table XVIII). Latencies

Table XVIII: Rapidity of Approach to a Source of Ultrasounds in a Y-Maze.

	Mean Latency in Y-Maze (sec.) (SEM)	
	Natural Ultrasound	Synthetic Ultrasound
Male	11.6 (1.0)	17.5 (2.6)
Estrous Female (Day 1)	9.7 (1.0)	10.7 (1.7)
Nonestrous Female (Day 3)	13.5 (1.4)	15.3 (2.0)

exhibited by estrous and nonestrous females differed significantly ($p = .048$, t-test for independent means) when natural ultrasounds, but not synthetic ultrasounds, were the discriminative stimulus. In each case, males exhibited latencies intermediate to and statistically indistinguishable from those of the two female groups.

Discussion

Results summarized in Tables XVII and XVIII show that high-frequency vocalizations such as those emitted by estrous female hamsters provide adequate directional and motivational cues to elicit rapid approach on the part of conspecifics of either sex. Among females, approach to a source of natural high-frequency calls is more likely and more rapid on the estrous day of the sexual cycle.

Hamster calls apparently are "attractive" to other hamsters. In this important sense, then, these signals seem well suited to serve as sexual attractants, facilitating social contact between conspecifics of opposite sex and appropriate endocrine state to engage in successful reproduction. These data add to other experimental data (e.g., Chapters V-VI) consistent with this interpretation. At the same time, however, the present results also suggest that there may be other functions for hamster ultrasounds. For instance, the mode of sexual attraction supported by these calls could differ from the most simple, one-female-attracting-one-male type, since other females are attracted to a source of estrous female ultrasounds. If high rates of ultrasound emission indicate that both an estrous female and a sexually mature male are present, other hamsters of similar endocrine conditions might approach with good expectation of locating a suitably primed individual of the opposite sex. Such a mechanism would be permitted by the suppression of female aggressivity seen previously to accompany sexual receptivity on estrous day (e.g., Chapter III). Finally, in addition to the above roles, hamster ultrasounds could have social significance totally outside the context of sexual reproduction.

EXPERIMENT 17: THE FACILITATION OF LORDOSIS BY TAPED ULTRASOUNDS

In earlier experiments (e.g., Experiments 15 and 16), attention has been focused on possible interpretations of the functional significance of female hamster ultrasounds. Sex differences in call structure (Chapter V) and stimulus determinants (Chapter VI) have suggested that male calls might play somewhat different roles in hamster social communication. For instance, a relatively low incidence of rapid amplitude and frequency changes in male ultrasounds suggests that they are more restricted in range than female calls and that some male calls might affect reproduction only after close social contact has been initiated (Chapter V). However, what aspects of male-female interactions might be susceptible to modulation by these male ultrasounds? We have investigated the effects of synthetic ultrasounds on the maintenance of lordosis, and have found that high-frequency sounds are capable of increasing the incidence and duration of lordosis by receptive females.

Method

Subjects and Test Procedures

Each of 11 estrous (Day 1) female hamsters was allowed 2 minutes of pre-exposure to the 41 x 20 x 31 cm plexiglas test arena. An intact adult male then was introduced and allowed to interact freely with each test female. Females generally exhibited lordosis with very short latencies. However, the stimulus male was permitted to interact with a test female for 60 seconds from her initial display of the lordosis posture. For 5 females, the male was removed at the end of this period and the duration of lordosis in the male's absence was noted. For the remaining six females, the same schedule was followed, with the exception that a playback of taped synthetic ultrasounds (see below) was begun 10 seconds prior to the male's removal and continued until the female first emerged from lordosis. Following this initial series of tests, each female was returned to her home cage for a period of approximately two hours. Then, a second series of identical tests was initiated, with females now assigned to the opposite experimental condition: individuals exposed to ultrasound playbacks during the first test were tested in silence during the second test series, and

those observed initially in silence were now exposed to ultrasounds throughout the post-male period. Thus, each individual female served as her own control, and the data of major interest consisted of the relative durations of lordosis in the presence versus the absence of recorded ultrasounds.

Procedures employed for the generation and reproduction of stimulus ultrasounds have been described in a previous section of this chapter. Participants in the present experiments were exposed to the usual 35 kHz sinusoids, in 100 msec. pulses, at constant rates of 1 call/second and at an intensity (near the center of the test arena) of approximately 55 dB SPL.

Results

Following brief exposure to a male, estrous female hamsters maintained lordosis for significantly longer durations in the presence of synthetic ultrasounds than in the absence of these cues ($p = .012$, sign test, see Table XIX).

Table XIX: Effects of Synthetic Ultrasound on the Maintenance of Lordosis in Estrous Females

Mean Duration of Lordosis (sec.) (SEM)	
Ultrasound	No Ultrasound
138.3 (31.5)	49.3 (9.0)
$p = .012$ (sign test)	

Informal observations suggest strongly that ultrasounds can affect the rapidity with which the lordosis posture is initiated, as well as its eventual duration. In fact, on numerous occasions, playbacks of natural or synthetic high-frequency sounds actually have induced lordosis by some estrous females deprived of any physical contact with a male. Less dramatic, but similar, effects upon lordosis latency may be of greater generality among estrous females.

Discussion

Artificially generated high-frequency sounds resembling natural hamster calls clearly can prolong the exhibition of the stereotyped posture of sexual receptivity (lordosis) by estrous females. Since the constant frequency and amplitude of our synthetic ultrasounds probably render them better mimics of male, than of female, vocalizations (see Chapter V), direct effects of synthetic ultrasounds on female lordosis suggest that hamster reproduction is facilitated by male vocalizations emitted during close social interaction. In particular, these findings suggest that high-frequency hamster vocalizations are well suited not only to facilitate the achievement of contact between an estrous female and a male, but also (a) to structure the resulting contact by stimulating lordosis by the female, and, (b) to insure that such contact will be maintained through a period of time sufficient to allow complete copulation leading to successful reproduction.

CHAPTER VIII. HAMSTER SOCIAL ORGANIZATION, SEXUAL BEHAVIOR AND AGGRESSION: A MODEL OF NONCONTACT COMMUNICATION

I. SUMMARY AND REVIEW: THE FUNCTIONAL SIGNIFICANCE OF HAMSTER ULTRASOUNDS

Factors which must be considered in evaluating the communicatory status of any natural sound include: (a) the physical properties of the signals; (b) the stimuli typically associated with changes in the rate of signal emission; and, (c) the manner in which conspecifics respond to the signal in question. Some attention to each of these issues seems crucial in determining if a signal possesses any communicatory value, and, later, in assessing the nature and specificity of any message encoded in the signal.

(a) We have found that hamsters of both sexes can emit pure ultrasounds (all components exceeding 20 kHz), but that sounds produced by males and females differ with respect to their physical characteristics (Chapter V). In particular, calls emitted by males include fewer abrupt changes in amplitude and frequency than female ultrasounds. Such sounds would be more difficult than those of females to localize over any distance, and might influence behavior at relatively close range. In fact, some male calls may be functional only following the achievement of initial social contact. Conversely, the rapid fluctuations in frequency and amplitude emphasized in female ultrasounds could facilitate the localization of a calling female over distances beyond the effective range of male calls. While limited information regarding hamster ecology and acoustic sensitivity obviates a precise estimate of the effective range of these vocalizations, we have suggested that some female hamster calls, emitted under ideal open-air conditions, might be audible over distances at least as great as 20 meters. While the information value of ultrasounds emitted at ground level might be attenuated much more severely and rapidly as a result of sound scattering by obstacles, hamster calls emitted within a closed burrow system might have effective ranges considerably greater than that estimated above. Clearly, naturalistic observations of hamster behaviors and ecology eventually will prove crucial for extremely

detailed interpretations of the functional significance of high-frequency hamster vocalizations. Nevertheless, our descriptions (see also Sewell, 1970; and, Sales, 1972a and b) of the physical characteristics of hamster calls do suggest at least that female hamster ultrasounds could be audible over moderate distances and, therefore, could serve effectively as "noncontact" signals advertising the location of a calling female.

(b) Female hamster ultrasounds encode information regarding reproductive state as well as location (Chapter VI). Basal (pre-male) rates of ultrasound emission by estrous females greatly exceed those typical of nonestrous females. Even higher rates of calling by estrous females may be provoked by cues normally indicative of a male's proximity. For example, receptive females may call "in anticipation of" routine exposures to stud males, or following the termination of brief heterosexual contact. In each case, olfactory or auditory cues provided by males may be instrumental in provoking increased ultrasound rates. For example, anesthetized males and male shavings are adequate to facilitate female calling. Similarly, playbacks of synthetic ultrasounds stimulate high rates of ultrasound emission by estrous females, possibly by mimicking the calling of nearby males (Experiment 15 of the previous chapter). These findings all are consistent with a view of female hamster ultrasounds as sexual advertisements which indicate the locations, receptivity and relative passivity of estrous females to male conspecifics. Since estrous females do not call during lordosis, female ultrasounds probably are infrequent during heterosexual contact and may operate primarily over moderate distances, attracting males to an initial rendezvous, or facilitating resumption of contact following accidental separation. The dependence of female ultrasound emission on olfactory and auditory cues suggests that these signals would be produced most frequently when a male is near and might be attracted, thus facilitating intraspecific contact while minimizing any attraction of potential predators.

Male hamsters, like females, respond to the presence of an opposite-sex conspecific with increased rates of ultrasound emission (Chapter VI). This effect is somewhat persistent, typically extending into the period immediately following removal of the stimulus female. Here, however, male call rates may decrease more rapidly than those of females, possibly indicating a greater degree of dependence of male vocalization rates on the immediate physical presence of female cues than in the opposite case. In turn, this suggests that high rates of male calling may be restricted to situations involving relatively close social contact, situations in which the direct facilitation of female reproductive behaviors might be as relevant a "goal" as localization over a distance.

At least in certain situations, male hamsters consistently discriminate between estrous and nonestrous females (Chapter VI). However, the differential responsiveness of males does not seem to have been based upon differences in olfactory cues provided by estrous and nonestrous females. While males do respond to female odor sources (anesthetized females) with increased ultrasound rates, effects elicited by estrous and nonestrous females are similar. Thus, some other difference in the stimulus values of females of different endocrine states must have accounted for discriminations exhibited by males following brief contact with awake females. Possibly the greater resistance, or aggressiveness, of nonestrous, as opposed to estrous, females (e.g., Chapter III) may tend to depress high male call rates ordinarily stimulated by exposure to female odors.

(c) In a simple choice situation (Y-maze), hamsters of both sexes are able to localize a source of ultrasounds, and are attracted to it. In more natural (and complex) settings, increased calling by an estrous female, such as seen in response to synthetic ultrasounds, might greatly facilitate her localization by the source of the "stimulus" ultrasounds. Once initial social contact has been achieved, male ultrasounds may facilitate the display of female reproductive behaviors in a manner quite analogous to the effect on lordosis duration exerted by synthetic ultrasounds.

The discussion above summarizes a variety of sorts of evidence consistent with the interpretation of male and female ultrasounds as communicatory signals which function, at least in part, to facilitate social contact leading to successful reproduction. Specifically, female vocalizations seem likely to act as sexual attractants over moderate distances, while distinct types of male calls may operate (a) over short-to-moderate distances to further aid in sexual attraction, and, (b) at very close range to facilitate the display of appropriate female reproductive behaviors. In addition to these roles in social communication, male and female ultrasounds could play other roles in both sexual and nonsexual communication among hamsters.

II. HAMSTER SOCIAL ORGANIZATION AND COMMUNICATION: A HYPOTHETICAL MODEL RELATING ACOUSTIC AND OLFACTORY SIGNALS

Golden hamsters of both sexes and most endocrine states are highly aggressive (e.g., Payne & Swanson, 1970; Chapter III). Violent fighting is the "rule" even within heterosexual pairs. Males may experience some chronic inhibition of aggressiveness toward females, possibly due to a hormone-dependent pheromone (Payne & Swanson, 1971b; also see Murphy & Schneider, 1970; Johnston, 1972; Payne & Swanson, 1972b; Murphy, 1973; Payne, 1974). However, females readily attack conspecifics of either sex except on their estrous days, when female aggressiveness is inhibited by ovarian estrogens and progesterone (Kislak & Beach, 1955; Tiefer, 1970; Chapter III). Together with other indirect evidence (see Chapter II), the extreme aggressiveness of male and female hamsters suggests that free-living individuals live solitarily, each possibly defending a "territory" surrounding a home burrow. Such a social organization, based on social intolerance and spatial separation, could raise problems concerning reproduction. In particular, having increased their separation to accommodate high levels of aggression, hamsters may depend upon specific communications which specify when and where males and females may find each other in order to reproduce. The detection and localization of a potential mate might be facilitated by distance communications. High-frequency acoustic signals are audible over short-to-moderate distances (Chapter V), and provide immediate

and relatively precise spatial information over this range. However, hamsters also exhibit stereotyped scent-marking behaviors ("flank-marking" and "vaginal-marking;" see Dieterlen, 1959 and Johnston, 1970a) associated with the deposition of at least two types of pheromones. The persistence of these olfactory cues (Johnston, 1974d) should allow them to serve as long-distance signals, acting at locations which are remote from the present location of the communicator and which could be dispersed over a relatively large area, such as an entire home-range. In fact, in a multimodal communication system, the greater persistence, but lesser localizability of hamster pheromones might neatly complement the shorter range, but more easily localized, high-frequency vocalizations. A hypothetical model describing one way in which olfactory and acoustic signals could act in concert to facilitate successful reproduction among hamsters is presented in Figure 31.

Levels of general activity, scent-marking and high-frequency vocalization by female hamsters fluctuate with the 4-day estrous cycle (a in Figure 31). Activity levels are significantly higher on estrous (Day 1) and proestrous (Day 4) days of the cycle than during diestrous (Days 2-3; Richards, 1966). Elevated levels of locomotion during proestrous might function normally to aid in the dispersal of vaginal-marks deposited during the flurry of vaginal-marking characteristic of Day 4 (Johnston, 1970a and b). In turn, the persistence of hamster vaginal discharge odors (Johnston, 1974d) could permit these signals to provide any recipients with advance notice of the resident female's impending estrous (Johnston, 1970a and b). Increased activity by estrous (Day 1) females also could facilitate hamster social communication leading to reproduction. Together with the elevated rate of ultrasound emission characteristic of estrous females (Chapter VI), increased locomotion through the occupied area could increase greatly the proportion of that area "swept" with spontaneous ultrasounds (and with other sensory modalities). In turn, this should increase the likelihood of our estrous female making vocal, or even direct, contact with a potential mate (possible "short-circuits" from point a to points i or 1 of Figure 31). The primary function of female flank-marking may be less closely related to reproduction than in the cases of vaginal-marking and ultrasonic vocalization. For example, rates of flank-marking by females in

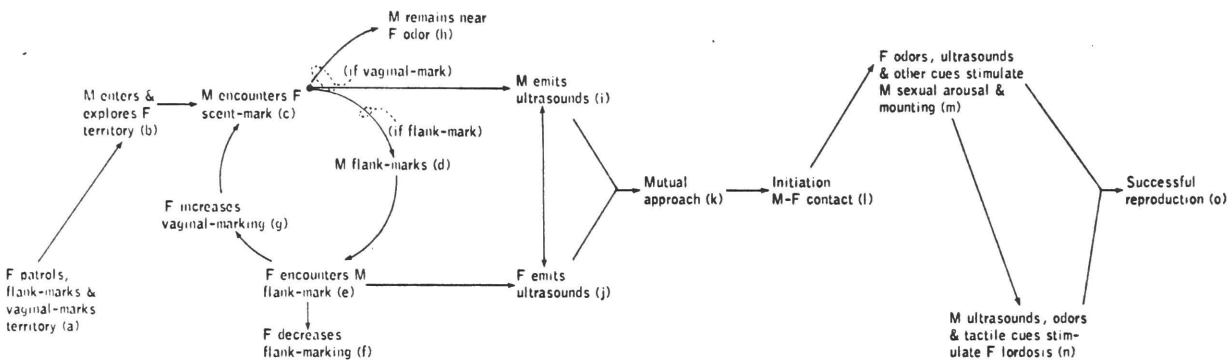


Figure 31. A hypothetical model of social communications leading to reproduction among golden hamsters. Male's behavior (M) above; female's (F) below. Lower case letters in parentheses code behavior elements for discussion in the text. Many of the "links" in this chain of social behaviors depend upon gonadal hormones. In particular, testosterone facilitates male responses at least at points d, h, m and o. Estrogen and progesterone facilitate female responses at least at points a, g, j, k, n and o. See text for details and references.

clean cages or male home cages tend to be cyclic (minimal on estrous day), but are generally much lower than the high, relatively constant, rates seen in the home cages of other females (Johnston, 1970a and b). This is consistent with other evidence suggesting that flank-marking rates are directly related to aggressive or territorial motivation, but are inversely related to sexual motivation and fear (Dieterlen, 1959; Johnston, 1969, 1970a and b, 1974a and c; Drickamer & Vandenbergh, 1973; Drickamer et al., 1973; Vandenbergh, 1973). Accordingly, flank marks are thought to serve primarily as territorial markers. Since the ability of a female to reproduce might depend on her ability to defend a territory, female flank-marking could contribute to reproductive success, at least in part, as a territorial "display."

Male and female European hamsters (Cricetus cricetus) live solitarily and defend territories surrounding their burrows (Eibl-Eibesfeldt, 1953). Males are permitted to enter the territory and burrow of an estrous female only for the brief period during which mating occurs. This suggests that reproduction among golden hamsters occurs during a visit by a male to the territory of a receptive female (b in Figure 31). Since male hamsters, like females, emit ultrasounds at low spontaneous rates (Chapter VI), a male intruder could make immediate vocal contact with the resident female, a possibility enhanced by the relatively high levels of activity exhibited by estrous females (possible short-circuit from b to j in Figure 31). Alternatively, the intruding male could contact the female directly (possible short-circuit from b to 1 in Figure 31). However, if hamsters occupy substantial home ranges (exceeding 16 x 20 feet), as suggested by Johnston (1970a and 1974c), then both of these relatively direct paths to male-female contact may be less likely than one mediated by male contact with a female scent-mark (c in Figure 31). The type of scent-mark encountered will depend, in part, on the resident female's reproductive state. The likelihood of encountering a fresh vaginal-mark probably will be highest on the female's Day 1, shortly after the cyclic burst of marking on Day 4 has been completed (Johnston, 1970a and b). The probability of contacting a vaginal mark also will be high on the female's Day 4, during her period of most intense marking behavior, but should be very low on Days 2 and 3

of the cycle. The attraction to female vaginal discharge exhibited by naive or experienced intact males suggests that a male contacting a vaginal mark will tend to approach and remain near that odor (h in Figure 31; see, Johnston, 1970a and 1974d; Murphy, 1973; Gregory et al., 1974). As a consequence, the male is likely to (1) remain in the female's territory, and, (2) approach other vaginal marks or other sources of vaginal-discharge odors. Each of these behavior-changes should increase the probability that the male eventually will contact the female during his visit. More immediate facilitation of male-female contact might be accomplished if males increased their rates of ultrasound production upon encountering a female vaginal mark. We have found that males do exhibit elevated ultrasound rates in response to female odors (Chapter VI). Since female vaginal discharge, but not flank-gland secretion, has been shown to stimulate other components of male sexual behavior (Johnston, 1972; Lisk, Zeiss & Ciaccio, 1972; Murphy, 1973), we hypothesize that female vaginal-discharge odors are responsible, in large part, for the stimulation of male ultrasound production by female odors (i in Figure 31).

A male hamster entering the territory of a neighboring female could make initial contact with a female flank-mark, instead of vaginal-mark (c in Figure 31). The probability of this is unclear in the absence of data describing rates of flank-marking by females in their home cages; however, the somewhat cyclic rates seen in clean cages and male home cages (Johnston, 1970a and b) suggest that fresh female flank-marks may be more prevalent on nonestrous days of the cycle. Nevertheless, contact with a female flank-mark probably will stimulate flank-marking by the male intruder (d in Figure 31: see Johnston, 1969 and 1974b). In contrast, a resident female encountering the flank-marks deposited by the male intruder (e in Figure 31) probably will decrease her own rate of flank-marking (f in Figure 31: see Johnston, 1970b). Other changes in female behavior consequent to contact with the male odor will depend on her reproductive state. In particular, a nonestrous female, especially if on Day 4 of the cycle, probably will increase her rate of vaginal-marking in response to male flank-gland scents (g in Figure 31: see Johnston, 1970a and b). In turn, this clearly will increase the likelihood that a male will contact these vaginal-marks (c in Figure 31),

decrease his own rate of flank-marking (Johnston, 1969 and 1970a), and eventually exit the loop depicted in Figure 31 (c - d - e - g) to move toward a more advanced stage in the chain of reproductive behaviors (e.g., h and i of Figure 31). More direct advancement toward male-female contact and successful reproduction might be achieved by an estrous female encountering male flank-marks (e in Figure 31). In particular, based on the ability of male odors to stimulate high-frequency vocalization by receptive females (Chapter VI), we suggest that male flank-gland scent can stimulate female ultrasound production (j in Figure 31).

Female ultrasounds can stimulate and guide approach by nearby males (Experiment 16). Conversely, the ability of natural female calls or synthetic ultrasounds to attract estrous females (Experiment 16) suggests that male ultrasounds can stimulate and guide approach by nearby females. Thus, ultrasounds by a hamster of either sex should elicit approach responses on the parts of nearby opposite-sex conspecifics. Furthermore, natural or synthetic ultrasounds can stimulate calling by receptive females (Experiment 15). Informal observations suggest that males, too, respond to playbacks of taped ultrasounds with increased rates of ultrasound production. Therefore, the end results of ultrasound production by either member of a male-female pair should be a rapid ultrasonic "chorus" involving both partners (arrow connecting i and j in Figure 31), together with mutual approach (k in Figure 31). Clearly, reciprocity in the stimulation of calling by one's potential mate, and in attraction toward the source of calls thus stimulated, should greatly facilitate the accuracy of approach, and thereby increase the likelihood that male-female contact will be initiated successfully (l in Figure 31). Once initial contact has been achieved, female odors (especially vaginal-discharge odors), ultrasounds and other cues probably help to decrease any male aggressiveness, and to stimulate male sexual arousal and properly oriented mounting behavior (m in Figure 31: see, Johnston, 1970a, 1972, 1974d; Murphy & Schneider, 1970; Doty, Carter & Clemens, 1971; Devor & Murphy, 1972 and 1973; Lisk et al., 1972; Murphy, 1973; and, Gregory et al., 1974). In turn, male ultrasounds (Experiment 17)

and odors (Lipkow, 1954; Dieterlen, 1959; Noble, 1972 and 1973), together with tactile stimulation resulting from male mount attempts (Murphy, 1974; Pfaff, Lewis, Diakow & Keiner, 1973), facilitate the display of lordosis by an estrous female (n in Figure 31). Prolonged lordosis by the female then permits the multiple mounts and intromissions by the male required for successful reproduction (o in Figure 31: see Dewsbury, 1972; and, Pfaff et al., 1973).

Various links in the behavior chain summarized in Figure 31 depend upon gonadal hormones. For example, testosterone has been shown to determine hamster flank-gland morphology and rates of flank-marking behavior (d in Figure 31: see Vandenbergh, 1971; Drickamer & Vandenbergh, 1973; and, Drickamer et al., 1973). The attraction of males to estrous female vaginal-discharge odors (h in Figure 31: see, Gregory et al., 1975), some male sexual behaviors (m in Figure 31: e.g., Beach & Pauker, 1949), male fertility (o in Figure 31) and probably other aspects of male reproductive performance also depend on androgenic stimulation. In the female hamster, clear fluctuations in levels of general activity (a in Figure 31: see, Richards, 1966), and in rates of vaginal-marking (a and g in Figure 31: see, Johnston, 1970a and b) and ultrasound production (j in Figure 31: see Chapter VI) with the normal estrous cycle suggest that these behaviors are modulated by some combination of ovarian estrogens and progesterone. The fact that Day 1 and Day 3 females consistently differed in their propensity to choose sources of ultrasounds in a Y-maze (Experiment 16) suggests that female approach responses toward male suitors (k in Figure 31) also are controlled by ovarian steroids. Even more direct evidence from hormone-replacement studies has implicated androgens in the regulation of female flank-marking and flank-gland morphology (a and f in Figure 31: see, Drickamer & Vandenbergh, 1973; and Vandenbergh, 1973), while ovarian estrogens and progesterone clearly are instrumental in the exhibition of lordosis by estrous females (n in Figure 31: e.g., Frank & Fraps, 1945) and in female fertility. Estrogen and progesterone also are responsible for the precipitous decline in female aggressiveness which permits the display of female sexual behaviors on estrous day (n in Figure 31: see Chapter III).

Thus, adequate gonadal hormone levels in the male and female hamster are required for the chain of communicative, courtship and copulatory responses to operate. In this way, steroid sex hormones synchronize behavioral with gametogenic preparations for successful reproduction. By the same token, the chain of behavioral responses amplifies hormonal effects so that variations in hormone levels can exert striking acceleratory or braking influences on the rate of behavioral progress toward copulation. When operating properly, the chain of coordinated male and female reproductive behaviors (Figure 31) should ensure that only reproductively competent conspecifics can complete the sequence.

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