Effects of vaginal–cervical stimulation in seven species of muroid rodents

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Summary. Six species of muroid rodents (*Peromyscus californicus*, *P. eremicus*, *P. gossypinus*, *Mesocricetus auratus*, *Rattus norvegicus* and *R. rattus*) were given artificial vaginal–cervical stimulation in an attempt to induce pseudopregnancy. Some females of each species became pseudopregnant, but it was not clear that stimulation patterns mimicking the copulatory patterns of conspecific males were more effective than other patterns of stimulation. In the non-domesticated species (*Peromyscus* and *R. rattus*) a few females responded to a variety of stimulus conditions, and no species differences in responsiveness among the wild species were evident. The established laboratory rodents (*R. norvegicus*, *M. auratus*) were markedly more responsive to artificial stimulation, suggesting possible effects of domestication on fecundity and reactions to handling. *Microtus ochrogaster*, an induced ovulator, ovulated in response to vaginal–cervical stimulation only if one intromission from a male was also provided. No other differences in the responses of reflex and spontaneous ovulators were apparent.

Introduction

Copulatory stimulation is critical for the initiation of neuroendocrine reflexes essential to the establishment of pregnancy in a variety of rodent species, and Diamond (1970) proposed the notion of a 'vaginal code' wherein the female is maximally responsive to patterns of stimulation that mimic the species-typical male copulatory pattern.

Laboratory rats (*Rattus norvegicus*), roof rats (*R. rattus*), golden hamsters (*Mesocricetus auratus*) and cotton mice (*Peromyscus gossypinus*) all display copulatory patterns in which a group of brief mounts, each with a single vaginal thrust/intromission, precede a mount with intromission and ejaculation. Several series of intromissions with terminal ejaculations occur in a typical mating session (Beach & Jordan, 1956; Beach & Rabedeau, 1959; Estep, 1975; D. V. Lovecky, personal communication). In contrast, cactus mice (*P. eremicus*), California mice (*P. californicus*) and prairie voles (*M. ochrogaster*) display multiple vaginal thrusts/intromission (Gray & Dewsbury, 1973; Dewsbury, 1974a,b). None of these 7 species exhibits locking during copulation, and all ejaculate several times in one copulatory episode. Only California mice and cotton mice have been observed to ejaculate at the first intromission, and only prairie voles are induced ovulators (Richmond & Conaway, 1969). Females of the species in which males display little persistence of copulatory activity and few ejaculatory series have low thresholds for pregnancy initiation relative to the females of species with more persistent males (Davis, Gray, Zerylnick & Dewsbury, 1974; Gray, Zerylnick, Davis & Dewsbury, 1974; Dewsbury & Estep, 1975; Estep, 1975; Lanier, Estep & Dewsbury, 1975; Davis, Gray & Dewsbury, 1976; Dewsbury & Lanier, 1976).

Pseudopregnancy, defined as a functional luteal phase in a non-pregnant cycle (see Conaway, 1971), can be initiated in laboratory rats by a wide range of patterns of artificial stimulation (Long & Evans, 1922; De Feo, 1966), but the effective stimulus parameters are more limited in golden hamsters (Diamond & Yanagimachi, 1968) and house mice (Diamond, 1970). Although mechanical stimulation can induce pseudopregnancy in *Microtus agrestis*, it is effective only in conjunction with copulation- or hormone-induced ovulation.

The purpose of the present research was to explore the range of stimulus patterns effective in

triggering ovulation and/or a functional luteal phase in females of various species. Artificial stimulation was used and the following features were considered: (1) degree of spontaneity of ovulation, (2) degree of domestication, (3) correlation of most effective stimulus with species-typical male copulatory pattern, (4) threshold related to persistence of male (see above), and (5) possible facilitatory effects of minimal copulatory activity.

Methods and Results

All females were laboratory born and reared and at least 90 days old. They were individually housed and had food and water available at all times. Rooms were maintained on reversed 16 h light:8 h dark photoperiods (14L:10D for cotton mice and hamsters), and artificial stimulation was applied during the dark phase. Daily vaginal smears were taken using a wire loop, except for hamsters which were examined externally only (Orsini, 1961). Artificial stimulation and/or mating was given on the day of pro-oestrus or oestrus (first day of virtual absence of leucocytes in the smear); for hamsters this was done on the day of transparent vaginal mucus. At least 2 regular vaginal cycles preceded artificial stimulation. Prairie voles, induced ovulators, were stimulated on the 2nd consecutive day on which they displayed an oestrous smear (over 50% cornified cells). Stimulation was applied either with a vibratory engraving tool (Sears, Roebuck & Co., Model 758.42960) fitted with a smooth, moulded 'penis' 3.8 mm in diameter or with the plunger from a 1.0 ml syringe, 4.5 mm in diameter. The tip of the stimulator was inserted to the cervix before vibration began.

Experiment I

Method. Ten cotton mice, 7 cactus mice and 3 California mice were stimulated with the vibrating engraving tool. Stimulus parameters were varied according to the typical copulatory patterns of the 3 species (see Table 1). Cotton mice and cactus mice each received 3 experimental treatments (2 cotton mice completed only 2 of these, all cactus mice served twice in each group): (1) one ejaculatory series with a male, (2) artificial stimulation, and (3) one ejaculatory series with a male plus artificial stimulation.

Results. Females were considered pregnant if a litter was born after 21–26 days or pseudopregnant if the vaginal smear remained dioestrous for at least 8 days (9 days for California mice). Each pattern of stimulation produced a low incidence of pseudopregnancy or pregnancy, but the response

Table 1. Number of copulations with a male, parameters of artificial stimulation used, and incidence of pseudopregnancy or pregnancy in *Peromyscus* species

Species	No. of series with male	No. of bouts	No. of insertions/bout	Duration of insertions (sec)	Interval between insertions (sec)	Interval between bouts (min)	No. pseudo-pregnant or pregnant
<i>P. gossypinus</i>	1	—	—	—	—	—	1/9
(cotton mice)	0	1	20	2	5	—	4/10
(N = 10)	1	1	20	2	5	—	1/9
<i>P. eremicus</i>	1	—	—	—	—	—	1/14
(cactus mice)	0	2	15	5	5	10	3/14
(N = 7)	1	2	15	5	5	10	2/14
<i>P. californicus</i>	0	2–5	1	30	—	60	4/10
(California mice)							
(N = 3)*							

*Two of these mice were stimulated 4 times, the other twice.

rate was <40% in all the treatments. Individual differences in responsiveness among females of a species contributed more to the variation than did the pattern of stimulation. The positive responses were contributed by 4/10 cotton mice, 2/7 cactus mice and 2/3 California mice, each of which responded 2–5 times. Additional attempts to induce pseudopregnancy in California mice by simulating multiple intromissions and multiple ejaculations were unsuccessful. Cactus mice were also tested with vibratory stimuli which simulated intravaginal thrusting and multiple ejaculations, cervical tapping without vibration, or one long continuous vibration, but no pseudopregnancies resulted. One of 5 *P. gossypinus* females which received simulation of multiple ejaculations became pseudo-pregnant, but other stimulus conditions were unsuccessful.

Experiment II

Method. Five female prairie voles (*Microtus ochrogaster*) in each of 5 groups were examined for the presence of corpora lutea (CL). In Group 1, voles received artificial stimulation (10 15-sec insertions with 5 thrusts/insertion, and 45 sec between insertions), and were examined on Day 3 after stimulation. In Group 2, voles received 15 sec of flank stimulation (Komisaruk, 1974) before each vaginal-cervical stimulation, which was as in Group 1, and were examined on Day 3. Females in Group 3 received one intromission from a male, no artificial stimulation and examination on Day 3. Group-4 females received one intromission from a male, artificial stimulation as in Group 1 and were examined on Day 3. In Group 5 females were treated as in Group 4, except that CL counts were made on Day 8 instead of Day 3. The reduction in CL number from Day 3 to Day 8 was taken as a measure of CL degeneration (Milligan, 1974). The engraving tool was used to stimulate the voles, but without vibration. Females were killed on the day of examination and CL from each ovary were counted with the aid of a dissecting microscope. Several ovaries were fixed in formalin, embedded in paraffin wax, sectioned at 10 μ m, stained with haematoxylin and eosin and examined microscopically to verify accuracy of CL counts.

Results. None of the 15 voles in Groups 1, 2 and 3 ovulated. Ovulation had taken place in 2 females in Groups 4 (5 and 6 CL) and 5 (2 and 5 CL). Corpora lutea in Group 5 females were fully developed, and no atypical luteal remnants were present. Thus, artificial stimulation with minimal stimulation from a male induced ovulation in 4/10 females, and the CL from these ovulations seemed to be functional.

Experiment III

Fifteen Syrian golden hamsters received 30 insertions, each with 5 sec of cervical 'tapping', using the syringe plunger (see Diamond & Yanagimachi, 1968). Twelve females (80%) became pseudo-pregnant (no post-oestrous discharge for 9–14 days).

Experiment IV

Method. The responses were compared of 32 F344/Mai laboratory rats (*Rattus norvegicus*) and 16 female roof rats (*R. rattus*), which were first-generation, laboratory-born offspring of wild-trapped animals. A single 10-sec vibratory vaginal-cervical stimulation was given with the engraver at different times, as shown in Table 2. An attempt was also made to mimic the male copulatory pattern: using the syringe plunger, 10 brief cervical taps were delivered with 1 min between stimulations and with a longer 10-sec stimulation 1 min later, to simulate ejaculation. After a 4-min rest period, an additional 3 series of 'intromissions' plus 'ejaculation' were given. Individual rats experienced 1–4 treatments but never the same treatment twice, and consecutive stimulations were separated by at least 2 weeks and 2 regular cycles.

Results. Laboratory rats showed a high incidence of pseudopregnancy in all groups, with maximal response to the pattern mimicking the male copulatory pattern ($\chi^2 = 7.9$, $P < 0.01$ compared with all the shorter vibratory stimulations). Roof rats responded only to the pattern mimicking the male pattern, but at a lower rate than laboratory rats ($\chi^2 = 5.6$, $P < 0.025$).

Table 2. Method (see text) and incidence of artificial induction of pseudopregnancy in *Rattus*

Species	Group	Stage of cycle	Time (h)	Method of stimulation	No. pseudo-pregnant/no. stimulated	Mean ($\pm$ S.E.M.) no. of days of dioestrus
<i>R. rattus</i>	1	Pro-oestrus	15.00	Vibration	0/5	—
	2	Pro-oestrus	21.00	Vibration	0/5	—
	3	Pro-oestrus	21.00	Tapping	3/7	11.0 $\pm$ 1.5
	4	Oestrus	10.00	Vibration	0/5	—
	5	Oestrus	15.00	Vibration	0/5	—
	6	Oestrus	21.00	Vibration	0/5	—
<i>R. norvegicus</i>	1	Pro-oestrus	15.00	Vibration	4/5	12.8 $\pm$ 1.8
	2	Pro-oestrus	21.00	Vibration	1/5	11.0
	3	Pro-oestrus	21.00	Tapping	7/7	14.9 $\pm$ 1.7
	4	Oestrus	10.00	Vibration	3/5	13.3 $\pm$ 1.5
	5	Oestrus	15.00	Vibration	3/5	13.3 $\pm$ 1.7
	6	Oestrus	21.00	Vibration	4/5	15.5 $\pm$ 0.3

Discussion

Artificial vaginal-cervical stimulation was effective in inducing pseudopregnancy or ovulation in some females of all the species examined, and there were no apparent differences between induced and spontaneous ovulators in susceptibility to artificial stimulation. The non-domesticated species responded considerably less to artificial vaginal-cervical stimulation than did hamsters and laboratory rats. The 'standard' laboratory rat has probably been selected for high fecundity, amongst other factors, and is possibly 'super-responsive' to reproduction-related stimulation (Price, 1967). However, Boice (1972) reports that selective trapping of submissive *R. norvegicus* accounts, in part, for the relatively lower reproductive success of wild-trapped animals of that species. Natural selection, as it is presumed to act in the wild, must to some extent still operate in laboratory-bred animals, as only the dominant animals reproduce successfully. An extreme reaction to handling, may partly account for reproductive failure, possibly by an adrenally mediated antigonadotrophic effect (Richter, 1949; Boice, 1972). The differences in response of the laboratory rats in the present study and those of De Feo (1966) probably reflect procedural differences of the 2 studies (light-dark cycle, time and day of stimulation). We tried various times of stimulation because the time of ovulation in *R. rattus* is not known.

There was no obvious greater response to stimulation patterns which mimicked those typical of the species. For example, *Peromyscus* females which became pseudopregnant responded to a variety of stimulation conditions; laboratory rats showed the greatest response to, and *R. rattus* females responded only to, simulations of the male pattern, although these amounts of stimulation were greater than those applied by vibration. While it seems likely, therefore, that species-specific patterns are more effective than stimulation unrelated to the male pattern in inducing physiological responses in the female, the present data are not conclusive.

There was no indication that the response threshold of the female to artificial stimulation was related to conspecific male persistence in mating: male laboratory rats indulge in prolonged copulation (Beach & Jordan, 1956) but some female *R. norvegicus* became pseudopregnant after just 10 sec vibratory stimulation. Similarly, in non-domesticated species of *Peromyscus*, females which showed a physiological response did so to a variety of stimulation conditions and levels. No consistent facilitatory effect of copulation on the response to artificial stimulation was apparent in *Peromyscus*.

Microtus ochrogaster, like *M. agrestis* (Milligan, 1975), responded to artificial stimulation only when it was preceded by one intromission from a male. It is possible that pheromonal or contact

factors are a necessary precondition to induced ovulation in these species or that different parameters of stimulation might give a higher incidence of response. Although 40% of *M. ochrogaster* ovulated after one intromission from a male when combined with artificial stimulation, and none did after the intromission alone, a 14% ovulation response to one intromission was reported in an earlier study (Gray *et al.*, 1974). More animals should therefore be examined to assess the relative importance of limited copulation and of artificial vaginal-cervical stimulation in the induction of ovulation in this species. *Microtus ochrogaster*, unlike *M. agrestis* (Milligan, 1974), showed no evidence of a dissociation between ovulation and the formation of functional CL. As prairie voles require less copulatory stimulation than *M. agrestis* for induction of successful pregnancy (Gray *et al.*, 1974; Milligan, 1975) another *Microtus* species with greater stimulation requirements is currently being examined in this context.

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