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Intromission Pattern and Species Vaginal Code in Relation to Induction of Pseudopregnancy

Abstract. *Mechanical stimulation was used to mimic normal vaginal stimuli during copulation in the mouse; the number and rate of intromissions were crucial influences on reproductive success. The best combinations for the mechanical induction of pseudopregnancy were comparable to those male behaviors normally seen during mating. The behavior of the male coincides with a species-related vaginal code, increasing chances for reproductive success between cospecifics.*

Observation of the copulatory behavior of many species, rodents in particular, reveals a definite series of intromissions interspersed with other sexual or nonsexual behaviors. With the report (*1*) indicating that the induction of pregnancy and pseudopregnancy in the hamster is related to the number and rate of intromissions, the hypothesis was formulated that the proper combination of the two would be crucial for successful reproduction in other species. Appreciation of such interacting factors would amplify the data for the rat. For the rat, Ball indicated that the number of intromissions per se is inconsequential for pregnancy as long as at least one or two ejaculations occur (evidenced by the finding of sperm plugs) (*2*). More recently, four or more intromissions, rather than three or fewer, are reported to significantly increase chances of pregnancy (*3*).

I have studied the combined effect of number and frequency of intromissions (insertions and interval between insertions) by means of mechanical induction of pseudopregnancy in the mouse. Heretofore, the induction of pseudopregnancy in this species has been impossible or rare without the use of an ejaculating vasectomized male (*4–6*).

Virgin DBA/2J strain mice obtained from the Jackson Laboratories (Bar Harbor, Maine) or bred in our laboratory were used. This strain was chosen because its copulatory behavior has been extensively studied (*7*).

All females were given freshly prepared ovulation-inducing gonadotropins. Pregnant mares' serum (PMS) [Equinex (Ayerst), serum gonadotropin; 2 international units] was administered at 4:30 to 5:30 p.m. followed 48 hours later by 3 units of human chorionic gonadotropin (HCG) ["A.P.L." (Ayerst), chorionic gonadotropin]. Between 9:00 and 11:00 a.m. on the day after administration of HCG, the females were vaginally stimulated so as to simulate intromission. Stimulation was with a mechanical vibrator (Vibro-Graver, Burgess Vibro Crafters, Inc., Chicago) fitted with a polished brass "penis" 3.5 mm in diameter. The penis rapidly moved back and forth (7200 strokes per minute) and was inserted up to the cervix. The day after stimulation was considered day 1. Until stimulation, the test females were kept in the colony room. After stimulation, the females were kept in a separate room without males to forestall pheromonal effects.

Various aspects of stimulation were tested (Table 1). The number (I) of insertions used was 3, 5, 10, or 15; the intervals between insertions (III) varied from 30 to 270 seconds. The median duration of intromission for males of this strain varies from 17 to 20 seconds; therefore, duration of each insertion (II) was standardized to last 20 seconds. The selection of these values was predicated on the extensive behavioral studies by McGill and his colleagues (*5*, *7*). The comparative efficiency of single prolonged stimuli and several other aspects of stimulation were also tested.

The effectiveness of stimuli in the induction of pseudopregnancy was evaluated in every case by a modification of the uterine decidual reaction technique (*8*). One uterine horn was traumatized by being extensively cut along the antimesometrial wall 3 days after stimulation (day 3); it was checked for a decidual reaction 3 days later (day 6). A decidual reaction was said to have occurred if the traumatized uterine horn weighed at least 50 percent more than the untraumatized horn and at least 90.0 mg (mean weight of the untraumatized control horn was 47.3 mg). Occasionally, ovaries were examined histologically for the prolonged maintenance of corpora lutea necessary for pregnancy and pseudopregnancy.

Throughout the experiment the mice were grouped one to four per clear plastic container (approximately 28 by 18 by 12 cm) with wood-chip bedding. The light cycle was maintained with darkness occurring from 6 p.m. to 6 a.m. Food and water were available as desired. The room was air-conditioned; temperatures varied from 65° to 75°F (18.3° to 24°C).

Mice can be mechanically induced to pseudopregnancy (Table 1). Consistent with our findings for the hamster (*1*), neither many insertions nor a single prolonged stimulation alone will insure the induction of pseudopregnancy. The successful stimulatory patterns appear to represent a key to a vaginal code which, within certain limits, is relatively specific for each species. Patterns of stimulation optimum for the induction of pseudopregnancy in the hamster (*1*) (I = 30, II = 5, III = 5) or capable of inducing 100 percent pseudopregnancy in the rat (*9*) (I = 1, II = 20) are relatively ineffective in the mouse. The pattern of stimulation sufficient to induce pseudopregnancy in the rat had been reported ineffective in the hamster and mouse (*4*). For the mouse, as for the hamster, the combination of factors most successful are those which resemble the normal mating pattern. McGill and colleagues (*5*, *7*) have reported that the median number of intromissions for this strain of mice varied in

different studies from 5 to 13 and the median time between intromissions approximated 90 to 215 seconds. I find a wide range of stimuli with patterns approximating these capable of effectively inducing the changes characteristic of pregnancy. For example, ten stimuli applied at 120-second intervals and 15 stimuli applied at 270-second intervals are highly effective in initiating pseudopregnancy (*10*). Significantly, a similarly long interval between intromissions is comparable to that seen in the normally copulating male.

In the variation of insertion number and the interval between insertions, the total insertion time (TIT) of vaginal stimulation and the total time of experimental exposure (TET) were also varied. These factors are comparable to the total intromission intervals and the total period allowed for mating. As seen in the hamster (*1*), prolongation of a single vaginal stimulation is not an efficient way to initiate pseudopregnancy. The maximum stimulus tested, of a duration comparable to the largest TIT (300 seconds), is not significantly more effective than shorter TIT's if the latter is the sum of spaced periodic insertions. The duration of the individual insertions must be considered too; even thirty 5-second insertions (TIT = 150) evenly spaced throughout 300 seconds (TIT = 5) were not too effective in inducing pseudopregnancy. The TET is also crucial; with multiple insertions, longer exposures seem better. These are comparable to the long median latencies to ejaculation (from 1376 to 1946 seconds) reported for this strain (*5*, *7*).

Naturally, normal mating is not structured so that only one pattern can insure initiation of pregnancy; indeed a wide combination of insertion numbers and intervals between insertions will suffice to induce maintenance of corpora lutea (Table 1). However, those stimulations that include multiple intromissions, that have more than 5 seconds between insertions, or that have extended exposure time will be relatively successful in inducing gestational changes. An increase in the number of insertions, the interval between insertions, or the TET will generally increase the chance for the initiation of pseudopregnancy until an optimum range of combinations is reached. Extremes of stimulation, however, do not necessarily increase the chances for induction of pseudopregnancy. Significantly, the so-called "vaginal code" seems flexible and depends upon the interaction between several independent variables among which are insertion number, rate of insertion, total exposure time, and duration of each insertion. Apparently some neural "comparator" or "integrator" is involved in the integration of these variables to affect the neuroendocrine reflexes. Undoubtedly other factors yet undefined are also integrated in a normal mating situation (*1*).

Table 1. Mechanical induction of pseudopregnancy in the DBA/2J mouse. Females were judged pseudopregnant if the traumatized uterine horn weighed more than 90 mg and more than 150 percent of the weight of the control. Abbreviations: I, number of vaginal insertions; II, duration of each insertion; III, interval between insertions; TIT, total insertion time; and TET, total duration of experimental exposure.

I (No.)	II (sec)	III (sec)	TIT (sec)	TET (sec)	Females made pseudo-pregnant (No.)	Females tested (No.)	Success (%)
1	20	0	20	20	2	14	14
1	50	0	50	50	1	6	17
1	100	0	100	100	2	7	29
1	200	0	200	200	4	9	44
1	300	0	300	300	4	8	50
3	20	30	60	150	6	8	75
3	20	60	60	240	2	6	33
3	20	120	60	420	4	10	40
3	20	180	60	600	2	6	33
3	20	270	60	870	3	6	50
5	20	30	100	250	4	7	58
5	20	60	100	400	2	8	25
5	20	120	100	700	4	10	40
5	20	180	100	1000	2	7	28
5	20	270	100	1450	5	8	63
10	20	30	200	500	1	6	17
10	20	60	200	800	4	9	44
10	20	120	200	1400	12	17	70
10	20	180	200	2000	5	11	45
10	20	270	200	2900	2	7	29
15	20	30	300	750	3	7	43
15	20	60	300	1200	3	7	43
15	20	120	300	2100	3	11	27
15	20	180	300	3000	2	8	25
15	20	270	300	4350	5	6	87
30	5	5	150	300	5	17	29
30	15	45	450	1800	2	3	67

Since the successful patterns of copulatory-like stimuli used here were based upon behavioral norms for this genetic strain, our success might be considered predictable; similarly consistent is the lack of success in other studies (*4*) in which were used vaginal stimuli which mimic the copulatory patterns of other species and which differ markedly from those of the mouse.

Land and McGill (*5*) have reported that in the mouse a large number of preejaculatory thrusts is neither necessary nor sufficient to induce luteal activity in the female. The ejaculatory reflex regardless of the number of thrusts, however, was considered crucial for the induction of pseudopregnancy (*6*). I would extend and modify these interpretations so that the number of intromissions be considered as a variable to be weighed. Furthermore, I would emphasize that, given sufficient stimulation appropriate to the species-related vaginal code, the ejaculatory reflex would not be necessary for the initiation of mechanisms which induce pseudopregnancy, such as maintained corpora lutea. This consideration is crucial for understanding mechanisms for luteotropin release and for studying artificial insemination in mice. If the definition of pseudopregnancy given by Land and McGill (*5*) is restated to encompass any prolongation of a normal 4- or 5-day ovarian cycle, even their data might be reinterpreted to show that 30 percent of 43 females were induced to pseudopregnancy by nonejaculating copulating males. My figures are then in keeping with theirs.

The stereotype in copulatory behavior seen in most animal species is hereby given significance. My results indicate that, within broad limits, a proper patterning of vaginal stimuli will induce maintenance of corpora lutea capable of supporting pregnancy.

Lincoln (*11*) has recently reported that in the rat there are specific hypothalamic neurons which receive sensory projections from areas apparently located in the region of the cervix and which are specific in their response to cervical probing. Aron *et al.* and Zarrow *et al.* (*12*) have demonstrated the quantitative interaction of copulatory stimuli and ovulation mediated by

gonadotropins. Adler (*13*) in a similar manner has demonstrated a relation between the number of intromissions and the probability of pseudopregnancy, and he, as well as Chester and Zucker (*14*), have shown that copulatory behavior is related to sperm transport and pseudopregnancy in the rat. These authors call attention to the need for comparative data. Studies of the hamster further indicate that release of progestin initiated by particular mating stimuli may be involved (*15*).

The concept is thus established that species-related neural stimuli from the vagina, integrated with other sensory inputs accompanying mating, are crucial for the initiation of neural and endocrine mechanisms supporting pregnancy and pseudopregnancy.

Note added in proof: Since preparation of this manuscript, McGill and Coughlin have suggested that the penile swelling of the ejaculating male leads to mechanical stretching of the vagina or cervix (or both) and that this is the stimulus which induces luteotropin release in the mouse (*16*).

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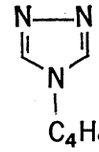
Wheat Leaf Rust: Control by 4-n-Butyl-1,2,4-triazole, a Systemic Fungicide

Abstract. *Compound 4-*n*-butyl-1,2,4-triazole was demonstrated as an enduring and selective systemic fungicide for the control of wheat leaf rust by foliar and soil applications. Among several species of rust fungi treated, only wheat leaf rust* (Puccinia recondita *Rob.) was controlled. Wheat stem rust, for example, was unaffected by either soil or foliar applications.*

The leaf rust disease of wheat incited by *Puccinia recondita* Rob. is apparent each year in the central and southern wheat-growing areas of the United States. The intensity of the infection is variable; however, a survey conducted over a 33-year period in Illinois concluded that leaf rust was the most damaging wheat disease in that region (*1*).

Chemical control practices for cereal rusts have been reviewed by Rowell (*2*). Chemical control of leaf rust has resulted in yield increases (*3*); however, maximum yield increases have been the result of multiple spray applications of protectant fungicides which act on the surface of the plant. An ideal method of controlling leaf rust would be the single application of an enduring systemic fungicide.

Several members of a series of 4-substituted 1,2,4-triazoles have shown enduring activity as fungicides by root uptake and as foliar sprays for control of wheat leaf rust. Superior performance has been noted for 4-*n*-butyl-1,2,4-triazole which has been extensively field tested under the code designation RH-124. The synthesis of 4-substituted 1,2,4-triazoles has been reviewed (*4*), and a recently described procedure may be utilized in preparing 4-substituted 1,2,4-triazoles (*5*).



4-*n*-Butyl-1,2,4-triazole

Soil incorporation of 4-*n*-butyl-1,2,4-triazole revealed a high degree of systemic fungicidal activity as a result of root uptake. Equal weights of clay-loam soil were spray treated in a rotary mixer to give 5.0 and 1.0 part per million of 4-*n*-butyl-1,2,4-triazole and of 2,3-dihydro-5-carboxanilido-6-methyl-1,4-oxathiin-4,4-dioxide; the latter compound was used as a standard treatment (*6*). The treated lots of soil were subdivided into two portions; one portion was seeded immediately after treatment with Pennoll wheat, and the second was kept moist and held for 12 days prior to planting. Inoculations were made by spraying a suspension of urediospores onto the wheat seedlings 7 days after each planting. Percentage of disease control was determined 14 days after each planting by counting lesions on ten seedlings in each of three replicates per dosage.

Table 1. Control of leaf rust of Pennoll wheat in laboratory and field tests by foliar application of 4-*n*-butyl-1,2,4-triazole. Field treatment was made at 372.8 liters of carrier per hectare to four plots of 1.5 by 1.6 m at the two-leaf stage. Inoculum was applied in the field 3 and 10 days after treatment as urediospores in talc. Control was determined by lesion counts on 25 plants per plot 35 days after treatment. Laboratory treatments were applied in a mixture of acetone and water (1 : 1). Inoculation was done in the laboratory test 7 days after treatment, and disease readings were made 15 days after treatment on the new growth. The standard treatment was 2,3-dihydro-5-carboxanilido-6-methyl-1,4-oxathiin-4,4-dioxide.

Treatment	Percentage control			
	Dosage (kg/ha)			
	0.14	0.28	0.56	1.12
	4-n-butyl-1,2,4-triazole			
Laboratory	88	97	100	100
Field			100	100
	Standard			
Laboratory	76	78	92	96
Field			0	9

Under these conditions, 4-*n*-butyl-1,2,4-triazole provided complete control of wheat leaf rust at 5.0 and 1.0 ppm, regardless of planting time. The standard treatment gave 40 and 0 percent control at 5.0 and 1.0 ppm, re-

Table 2. Summary of the spectrum of fungicidal activity of 4-*n*-butyl-1,2,4-triazole. Minus indicates not active; plus, active; and zero, not tested.

Pathogen	Host	Root uptake (10 ppm at planting)	Foliar spray (1200 ppm)
Uromyces phaseoli	Bean	—	—
Puccinia coronata	Oats	—	—
Puccinia recondita	Wheat	+	+
Puccinia graminis	Wheat	—	—
Puccinia hordei	Barley	—	0