tion was considered day 0 of gestation.

Chloramphenicol (CAF hemysuccinate, solution containing 50 mg of chloramphenicol per milliliter) was administered to the pregnant mothers or to the newborn pups. For the rats in group A, 50 mg of chloramphenicol per kilogram of body weight per day was injected subcutaneously into the mothers from days 7 to 21 of gestation. Since chloramphenicol passes freely through the placental barrier, one can assume that the fetuses received roughly the same dose as the mothers. For the rats in groups B and C, the chloramphenicol, 50 or 100 mg/kg-day, respectively, was injected subcutaneously into the newborn pups for the first 3 days after birth. The rats in group D served as controls; both the pregnant rats and their young received subcutaneous injections of saline.

Weight gain during pregnancy, litter size, fetal weight, gross malformations of the fetuses, and weight gain of the offspring were recorded. After birth the litter size was adjusted to eight to ten pups in order to maintain a standard nutritive status, equal numbers of each sex being left when possible.

At the doses used, chloramphenicol did not affect the course of pregnancies, litter size, fetal weight, or postnatal weight gain; furthermore, no gross malformations were noticed in the offspring of rats treated with chloramphenicol during pregnancy, and there was no mortality in the groups treated both before or after birth.

At weaning, rats from each group were divided into two subgroups of males and females and, when they were 60 days old, ten rats were selected at random from each of the eight subgroups for the avoidance learning study.

Shuttle boxes divided into two equal and communicating compartments were used. The conditioned stimulus was the sound of a buzzer: if the rat did not cross the passage between the compartments within 5 seconds, the unconditioned stimulus (an electrical shock of 25 V, 1.8 mA) was delivered through the grid floor of the box. Ten consecutive trials at 40-second intervals were performed daily (in the morning, from 0800 to 1200) for 20 consecutive days.

As shown in Fig. 1, the administration of chloramphenicol both during fetal and neonatal life impaired the acquisition of a conditioned avoidance response. The difference between treated and control animals was highly significant in all cases, although it was more marked in males than in females. The pain threshold was evaluated (hot plate test, temperature of the plate 55.5°C) before we conducted these behavioral tests, and no significant differences were observed among the eight groups (mean values ranged from 4.90 ± 0.43 to 6.15 ± 0.79 seconds).

Since chloramphenicol selectively inhibits the protein synthesis in the brain junctional complexes of mammals (*1*), reduced synaptogenesis might play a role in the learning deficit that we report here. One cannot exclude the possibility that the antibiotic interferes with neuron or glial production or migration, or with other aspects of brain differentiation. Metabolic or endocrine changes are other possible mechanisms for the observed effects.

Like other agents described as "pure behavioral teratogens" (*2*), chloramphenicol induces abnormalities in the behavioral capacities of the offspring, unaccompanied by weight loss, mortality, or gross malformations.

Although we cannot extrapolate the present results to humans, the finding that chloramphenicol impairs the acquisition of a conditioned avoidance response in rats should, in our opinion, induce clinicians to be even more cautious in the use of this antibiotic during pregnancy and in infancy. The doses we used are not far from those given to patients (25 to 50 mg per kilogram per day).

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Fetal Female Rats Are Masculinized by Male Littermates Located Caudally in the Uterus

Abstract. *Female rats are masculinized in utero by male littermates sharing the same uterine horn. Increased anogenital distances in neonatal females and mounting behavior in adult females are related to the presence of males on the caudal side of the females in the uterine horn. Contrary to current beliefs, interamniotic diffusion may not be responsible for the exchange of masculinizing agents among fetuses. Since uterine blood flow in the rat is from the direction of the cervix toward the ovary, masculinizing hormones secreted by fetal males may be carried via the uterine vasculature to female littermates located further downstream.*

Normal female rats occasionally display male-like mounting patterns (*1*). Indeed, under various treatment conditions some female rats show the motor pattern of ejaculating males (*2*). The capacity of normal females to exhibit male sexual behavior apparently depends on prenatal exposure to androgen, since such behavior potentials are reduced markedly by prenatal treatment with antiandrogenic drugs (*3, 4*).

A major source of androgen in fetal females is believed to be the male littermates. Female rats developing between two males in utero have longer anogenital distances (an androgen-sensitive morphological measure) at birth and higher frequencies of mounting in adulthood than females located three positions from the nearest male (*4, 5*). Clemens (*5*) proposed that interamniotic diffusion of androgen from contiguous male fetuses masculinized neighboring females. Simple interamniotic diffusion should yield gradations of masculinization in direct proportion to distance from males in utero, but equivalent masculinization occurred in females separated from the closest male by another female and those contiguous to one male (*4, 5*). This discrepancy suggested that diffusion across adjacent amniotic membranes might not be the mechanism of intrauterine exchange of androgen among fetuses. An alternative mechanism is offered by the uterine vasculature.

The rat has a separate vascular system for each uterine horn, with both the arterial and venous flow proceeding from the cervical end toward the ovary (*6*). The uterine vein and artery in the rat parallel one another and are in close apposition (Fig. 1). This organization of the uterine vessels prompted the suggestion that the luteolytic effect exerted by the uterus on the ovary in the rat may be mediated by substances that pass directly from the venous drainage into the arterial supply (*6*). Using a similar line of reasoning, we proposed that the venous drainage from male fetuses introduces substantial amounts of androgen into the

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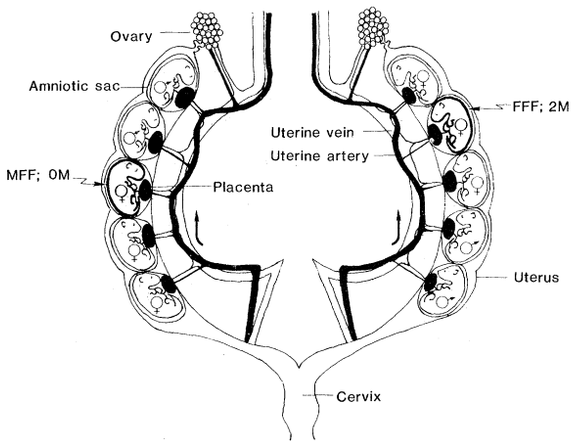

Fig. 1. Ventral view of the uterine horns and accompanying vasculature of a pregnant rat. The arrows indicate the direction of blood flow in the ovarian vein and artery. One female in each uterine horn is classified according to the contiguity and caudal male methods. We would predict that the MFF–0M female would be less masculinized than the FFF–2M female. (By the ovarian male method, the MFF–0M female would be classified M2 and the FFF–2M female classified M0.)

venous circulation of the uterus. The proximity of the uterine vein and artery may allow hormones to pass into the arterial flow that supplies blood to fetuses located downstream from androgen-producing males. If these fetuses are females, partial masculinization of androgen-sensitive tissues should occur. The present study supports this hypothesis; that is, the relative location of male and female fetuses with regard to the direction of flow within the uterine vasculature is a better predictor of masculinization of female rats than is contiguity per se.

The female offspring of 17 Sprague-Dawley rats (Madison, Wisconsin), 67 to 76 days old at the time of mating, were studied. Animals were housed in a temperature-controlled vivarium (about 23°C), maintained on a reversed cycle of 12 hours of light and 12 hours of darkness (lights out at 8 a.m.).

The animals were mated between 3 and 4 p.m. The day of impregnation was defined as day 0 of gestation. All litters were delivered by cesarean section on the morning of day 22 of gestation (*7*). The female was killed by cervical dislocation, and the uterine horns were removed intact through a midline incision in the abdomen. The pups were removed individually, laid out according to uterine position, cleaned, and weighed to the nearest 0.01 g. Anogenital distances were measured to the nearest 0.04 mm from the posterior base of the phallus to the anterior edge of the anus by use of a dissecting microscope with a micrometer eyepiece. Each pup was identified by injecting India ink into a paw; the pup was nursed by a newly parturient rat whose litter had been removed. The female offspring were weaned at 23 days of age and housed two or three per cage. A second experimenter marked the ear of each animal with a number not corresponding to the paw mark; this was done to ensure that the investigator scoring behavior would have no knowledge of the uterine position.

All females were ovariectomized bilaterally at 60 to 65 days of age. One week after surgery, the animals were given 10 μg of estradiol benzoate intramuscularly, and 43 hours later 1.0 mg of progesterone. Behavioral testing did not accompany the first series of hormone injections. This hormone regimen of a single injection of estradiol benzoate followed by progesterone was given once a week for the next 3 weeks; a test of female behavior was conducted 5 hours after progesterone administration. Behavioral testing consisted of allowing a sexually active male to adapt to the testing arena for 5 minutes and then introducing an experimental female. The test was terminated when the female had received ten mounts. A lordosis quotient (the number of lordoses divided by the number of mounts, times 100) was calculated for each of the three tests.

Tests for masculine behavior were initiated 2 weeks after the tests for female behavior. Each experimental animal was allowed a 5-minute adaptation period to the arena, after which an estrous female (*8*) was admitted for 20 minutes. Mountings accompanied by pelvic thrusting were recorded. After the first test, all females received daily injections of 250 μg of testosterone propionate for 14 consecutive days. Tests for mounting behavior were given every 3 or 4 days during testosterone treatment, for a total of four tests; these tests and the one given before testosterone treatment resulted in five tests for each animal.

The data were grouped in three ways (Fig. 1). The contiguity classification (*4, 5*) was used to assign animals to one of the following groups: females that developed in utero directly between two females (FFF), females that developed between one male and one female (MFF), and females that developed between two males (MFM). The caudal male classification grouped females according to whether they had zero (0M), one (1M), or two or more (2M) males located caudal (upstream to the direction of uterine blood flow) to themselves in the uterine horn. Finally, the data were grouped according to the number of males located on the ovarian (downstream) side of the female (M0, M1, or M2). The caudal and ovarian classification systems did not require that males be adjacent to the female (*9*).

Fisher tests were performed on the proportion of females responding with male behavior (*10*) in each of the three classification groupings. A definitive pattern emerged when the data on masculine behavior were organized according to the number of males on the caudal side (Table 1). A smaller percentage ($P < .05$) of 0M females mounted than either 1M or 2M females. There was a significant difference in frequency of mounting among 0M, 1M, and 2M females ($H = 6.5$, corrected for ties; $P < .04$) (*11*). Comparisons between groups showed that the frequency of mounting was lower for 0M females than it was for the 1M (Mann-Whitney U test; $P < .03$) and 2M ($P < .05$) groups; 1M and 2M females did not differ. Classifying females according to the number of males located on the ovarian side yielded no significant differences on any measure of male behavior.

Analysis by the contiguity classification showed that a higher proportion of MFM than FFF females mounted ($P < .04$), but no other comparison was significant. Although the frequency of mounting appears to be lower for the FFF group than it is for the MFF and MFM groups, differences between groups were not statistically significant ($H = 1.1$, corrected for ties), possibly because of the low power of the statistical comparisons that resulted from the small number of responding FFF animals. Two-way analyses of variance (days by uterine position) showed no significant differences within any classification system (*12*) for the mean lordosis quotients (Table 1).

Morphological measures obtained at the time of delivery (Table 1) showed significant differences in body weight when the females were classified according to the number of males located on the caudal side of them in the uterine horn ($F = 8.384$; d.f. = 2, 89; $P < .001$); 0M females were heavier than females in both the 1M (Newman-Keuls test; $P < .01$) and 2M ($P < .05$) groups. The

Table 1. Measures of lordosis quotient (LQ) and mounting behavior in female adults and of anogenital distance (AG) and body weight (BW) taken on the day of delivery. Values are means ± standard error. Percentage mounting indicates females exhibiting two or more mounts in all tests of masculine behavior. Differences in sample size between female behavior and masculine behavior tests reflect attrition because of mortality. The caudal male classification designates females with zero (0M), one (1M), or two or more (2M) males located caudal, but not necessarily adjacent, to them in the uterine horn. The ovarian male classification designates females with zero (M0), one (M1), or two or more (M2) males located on the ovarian side of them in the uterine horn. The contiguity classification designates females flanked by two females (FFF), by a male and a female (MFF), or by two males (MFM) in the uterine horn.

Group	*N*	LQ	*N*	Percentage mounting	Mounts (No.)	*N*	AG (mm)	BW (g)	AG/BW (mm/g)
					Caudal male				
0M	24	88 ± 3	22	32	7.9 ± 3.0	37	1.06 ± 0.02	5.82 ± 0.09	0.183 ± 0.004
1M	9	98 ± 1	8	75*	22.0 ± 9.4†	26	1.02 ± 0.02	4.94 ± 0.23‡	0.215 ± 0.008‡
2M	16	92 ± 4	15	67*	24.4 ± 7.4*	29	1.07 ± 0.02	5.35 ± 0.15*	0.207 ± 0.006‡
					Ovarian male				
M0	13	93 ± 3	12	50	15.3 ± 6.3	28	1.07 ± 0.02	5.45 ± 0.17	0.201 ± 0.007
M1	25	90 ± 3	23	52	16.3 ± 5.1	42	1.03 ± 0.02	5.28 ± 0.16	0.202 ± 0.006
M2	11	92 ± 5	10	50	15.5 ± 7.6	22	1.08 ± 0.03	5.64 ± 0.13	0.193 ± 0.006
					Contiguity				
FFF	13	91 ± 3	12	33	4.5 ± 2.5	20	1.00 ± 0.03	5.65 ± 0.21	0.181 ± 0.007
MFF	20	88 ± 4	19	42	18.9 ± 6.3	39	1.08 ± 0.02	5.27 ± 0.16	0.210 ± 0.006*
MFM	8	97 ± 2	7	86*	19.3 ± 8.9	15	1.05 ± 0.02	5.44 ± 0.15	0.194 ± 0.007

*Significantly different from 0M or FFF group, $P < .05$. †Significantly different from 0M group, $P < .03$. ‡Significantly different from 0M group, $P < .01$.

latter groups did not differ significantly from each other (*13*). Direct contiguity of male littermates did not significantly affect body weight. Although there were no significant group differences in anogenital distance within any of the classification systems, body weight and anogenital distance were correlated significantly ($r = .407$; $P < .001$). To obtain a measure of anogenital distance that was not confounded by group differences in body weight, the ratio of anogenital distance to body weight was computed for each animal. This yielded a significant effect on the classification by the number of males on the caudal side ($F = 6.998$; d.f. = 2, 89; $P < .01$); the ratio of anogenital distance to body weight was smaller in the 0M females than in either the 1M or 2M group ($P < .01$). The latter groups did not differ. Contiguity to males also was significant ($F = 3.971$; d.f. = 2, 7l; $P < .025$) in that MFF females had higher ratios of anogenital distance to body weight than FFF females (Newman-Keuls test; $P < .05$), but no other group comparisons were significant. There were no significant differences on any measure of morphology if the data were grouped according to the number of males on the ovarian side.

There was considerable overlap in the composition of the FFF and 0M groups (75 percent of FFF females belonged to the 0M group). Similarly, females in the 1M and 2M groups contributed heavily (87 percent) to the composition of the MFM group. The MFF females, however, were distributed among all three of the caudal male subgroups, and this group is therefore particularly important in determining the relative merits of the two mechanisms proposed to mediate exchange of hormones among fetuses. While every female in the MFF group was directly contiguous to one male in utero, they differed in whether any males were located on the caudal side. We found that MFF females with no males caudal to them had lower ratios of anogenital distance to body weight ($t = 2.212$; d.f. = 37; $P < .05$) on day 1 ($N = 19$; mean = 0.196 ± 0.007) than did females with one or more males caudal to them ($N = 20$; mean = 0.223 ± 0.010). In addition, the frequency of mounting for MFF females with a male on the caudal side ($N = 7$; mean = 33.3 ± 13.7) was higher (Mann-Whitney *U* test; $P < .05$) than that for MFF females with a male only on the ovarian side ($N = 12$; mean = 10.5 ± 4.9).

This study supports reports (*4, 5*) that the morphology and adult behavior potentials of female fetuses can be masculinized by hormones released by males sharing the uterus. The masculinizing agent, although not identified in the present study, is presumed to be testosterone (*14, 15*). Contiguity of male and female fetuses is not sufficient to produce masculinization of females. Intrauterine masculinization of females requires the presence of a male caudal to females within the same uterine horn. A single male on the caudal side appears to be as effective in inducing masculinization as are two or more.

Female mice located between two males have longer neonatal anogenital distances, exhibit later vaginal opening, are more aggressive, mark with urine at a higher rate, and have higher fetal testosterone titers than females developing between two females (*15, 16*). These findings are consistent with the mechanism we are proposing, since there is considerable overlap when females are classified as belonging to the FFF and 0M groups or the MFM and 1M or 2M groups. The MFF group is critical for our theory, since it contains females with and without males caudal to them. Our study indicates that the morphology and behavior of females with a contiguous male on the ovarian side but no males on the caudal side closely resemble those of FFF females, whereas MFF females with males caudal to them, even when they are not contiguous, are as masculinized as the MFM group (*17*). Since the amniotic sacs of MFF females in these two categories are in equally close contact with the amniotic sacs of males, the masculinizing agent cannot be passing via diffusion through amniotic membranes. The exchange may be effected through the proximity of the venous and arterial vessels that serve the uterus and the fetuses.

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7. The gestation period for this strain is 23 days.
8. Lure females had been ovariectomized and were brought into behavioral estrus by 10 μg of estradiol benzoate and 1.0 mg of progesterone injected intramuscularly 48 and 6 hours, respectively, before behavioral testing.
9. Since the last female on the ovarian end of the uterine horn had only one adjacent littermate, the data from these animals were excluded from the contiguity classification. However, these females were included in the analyses based on the number of males located on the caudal or ovarian side of the female. This accounts for the differences in total sample size, given in Table 1 for the three methods of classification.
10. Responding females are those exhibiting two or more mounts during the five tests for male copulatory behavior.
11. Comparisons of the average number of mounts exhibited by females in the several groups were made with Kruskal-Wallis tests. Parametric statistics could not be used because of the large number of zero scores in the FFF and 0M groups.
12. Since the lordosis quotient is a proportional measure, the analyses of variance were performed on scores transformed according to the formula $x' = 2 \arcsin\sqrt{x}$ [B. J. Winer, *Statistical Principles in Experimental Design* (McGraw-Hill, New York, 1971)].
13. Fetal body weight apparently is determined by a number of interacting variables. Although male rat fetuses tend to be heavier than their female littermates, this sex difference appears as early as day 12 of gestation [W. J. Scott and J. F. Holson, *J. Embryol. Exp. Morphol.* **40**, 249 (1977)]. Since the gonads differentiate after this time, it is unlikely that the difference is due to the action of fetal androgens. Furthermore, the female offspring of mothers injected with testosterone propionate or androstenedione during pregnancy show reductions in body weight that persist into adulthood [(*3*); I. L. Ward, *Horm. Behav.* **1**, 25 (1969); H. B. Popolow and I. L. Ward, *J. Comp. Physiol. Psychol.* **92**, 13 (1978)]. Paradoxically, prenatal exposure to antiandrogens has no effect on the birth weight of female rats (*4*). These conflicting data make it difficult to interpret the differences found in the birth weight of females classified according to their location in utero relative to male littermates. However, in agreement with our data, Clemens (*5*) reported that females derived from all-female litters had higher birth weights than females developing among male littermates.
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17. Within the combined 1M and 2M groups of the caudal male classification system, there were approximately equal numbers of females in uterine positions 2 through 6 (1 being the position closest to the cervix). These females did not show any significant morphological or behavioral differences when they were classified according to absolute uterine position. Thus, it is unlikely that a general nutritional gradient or any other variable related primarily to absolute position in the uterine horn, independent of the locations of males, had any important influence. On the other hand, secretions from the maternal ovary might have localized effects on female fetuses occupying positions close to the ovary. This possibility is not supported by the ovarian male classification data presented in Table 1. Furthermore, a comparison between females located immediately adjacent to the ovary and all other females indicated no statistically significant effect on any measure of morphology.
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Reformation of Organized Connections in the Auditory System After Regeneration of the Eighth Nerve

Abstract. *Binaural cells in the superior olive normally have identical frequency sensitivities when acoustically stimulated via either ear. The precision with which central connections are reformed after auditory nerve regeneration can be determined by comparing the frequency sensitivities of the two binaural inputs to these cells. Three months after cutting the nerve and subsequent regeneration in the leopard frog, binaural cells once again have well-matched frequency sensitivities. Thus, the specificity of central connectivity that characterizes the auditory system in normal animals is restored after regeneration.*

A striking feature of the vertebrate nervous system is the specificity with which connections between neurons are made (*1*). The auditory system is exemplary in this regard. Each auditory nerve fiber is "tuned" so that at low stimulus intensities it will respond to a tone of a characteristic frequency, namely the fiber's best excitatory frequency (BEF), whereas at higher tonal levels it responds to a broader frequency band. The threshold of the fiber, plotted as a function of tonal frequency and intensity, represents its tuning curve and is consequently V shaped. Within the vertebrate central auditory system at least up to the midbrain, many neurons maintain V-shaped excitatory tuning curves (*2*), which are almost as narrowly tuned as those in the auditory nerve itself (*3–6*). This preservation of frequency selectivity suggests that nerve afferents with very different BEF's do not innervate the same central cell. Binaural cells, which receive input from both ears, provide further evidence of precise interconnections within the central auditory system. In response to tones, binaural cells, whether excited by both ears (E-E) or excited by one ear and inhibited by the other (E-I), usually exhibit similar best frequencies for both ears (*4, 6*). Thus, similarly tuned afferents from both sides of the brain must converge systematically onto a common target cell.

To gain insight into how these highly precise connections may be made, we have developed a preparation based on the regenerative properties of auditory nerve fibers in anurans (frogs and toads). The VIIIth nerve of amphibians, unlike that of mammals (*7*), is able to regenerate back into the central nervous system after its fibers have been severed (*8*). We have exploited this phenomenon to study the characteristics of tuning curves of central auditory neurons after reinnervation. It is critical to know whether the regenerating afferents return to specific cells, or whether the afferent fibers determine the innervation pattern among themselves, independently of the identity of the postsynaptic cells. This question can be approached by studying the similarity of the best frequencies from the two ears for binaural cells. Since most binaural cells in the anuran's central auditory system have similar frequency sensitivities when stimulated by either ear (*5, 6*), the tuning curve derived from the side with the intact nerve can serve as a marker to indicate the best frequency for the original innervation. If, after reinnervation, binaural cells have matching best frequencies again, it would suggest that the regenerating afferents have remade contact with their former postsynaptic cells. On the other hand, if the best frequencies of the binaural cells are largely mismatched, regenerating fibers would seem not to have returned to their original target cells within the dorsal medullary nucleus, but to have sorted out independently of the identity of the postsynaptic cell.

For our electrophysiological study, we selected the superior olivary nucleus, a second-order auditory nucleus, as our recording site. In anurans this nucleus receives its dominant excitatory input from the contralateral dorsal medullary nucleus, the presumed homolog of the mammalian cochlear nucleus, which in turn receives input from its ipsilateral VIIIth nerve (*6, 9*) (Fig. 1A). Binaural cells are reliably encountered in the anuran's superior olive (*6*). Adult leopard frogs (*Rana pipiens*) were anesthetized with MS-222, and the VIIIth nerve on one side was exposed through the roof of the mouth. With a tungsten microhook, the nerve was totally severed from the brain at the point where it penetrated the medulla. The animals were then housed in individual tanks at 20°C and allowed to recover for 3 months or longer. No attempt was made to control the acoustic environment. Ambient noise levels in the recovery tanks were generally around 72 dB sound pressure level (relative to 20 $\mu N/m^2$) and included energy from 50 Hz to 4kHz, which encompasses the frequency range of hearing of this species (*10*).

During recording, the animals were anesthetized with Nembutal (sodium

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