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9. Operated jointly at the time by the U.S. Naval Ordnance Test Station and the U.S. Missile Center. Since January 1965, the facility has been operated by the U.S. Naval Missile Center.
10. The frequency response of the acoustic link was assumed to be flat within 2 db out to at least 16 kcy as specified by the manufacturers of the various equipment components. The fidelity of the recorded signals, however, was somewhat lower since the signals from the receiving hydrophones were not fully compensated for frequency roll-off before recording. The receiving hydrophones were Chesapeake ceramic-type, model SB-1546 with a frequency response of ±3 db to 5 kcy with a roll-off of 10 db per octave above 5 kcy. The transmit hydrophones were Massa barium titanate TR-14A with a linear frequency increase of 6 db per octave from 1 to 30 kcy. Nortronics model PL 100 amplifiers were used with a frequency response of ±2 db from 0.02 to 16 kilocycles at the neutral setting of the tone equalization adjustment. (The tone equalization adjustment was set to amplify the higher frequencies in order to compensate for the 4 db per octave difference between the transmitting and receiving hydrophones.) The amplifiers were set sufficiently low to eliminate feedback squeal and still permit the animals to hear each other. The impedance-matching transformers were Triad model H. S. ND-181 and were distortion-free for frequencies up to 20 kcy. The signals were recorded on a UHER model 8000 stereo magnetic tape recorder with a frequency response at 190.5 mm/sec of ±3 db from 0.05 to 20 kcy with wow or flutter less than ±0.15 percent. The generator for dash-dot and dot-dash signals, designed and constructed by Dr. C. Scott Johnson of the U.S. Naval Ordnance Test Station, drove two University model MM2F underwater speakers.
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Genotype and Prenatal and Premating Stress Interact To Affect Adult Behavior in Rats

Abstract. *Open-field ambulation scores of rats were affected by stress received by their mothers prior to mating, whereas avoidance-conditioning scores were affected only by gestational stress. The direction of effects on ambulation depended upon offspring genotype, while those depending on conditioning were unidirectional. Both effects were mediated prenatally by the mother.*

Offspring behavior can be affected by the prenatal experiences of the mother. Differences in the effects on their offspring of prenatal treatments of female rodents dependent on the mothers' strain and on the sex of the offspring focus attention on the importance of genetic variables in determining such differences (*1–3*) and suggest that differential susceptibility of either the mother or the fetus may account for them. But some experiments on prenatal stress fail to exclude the effects of premating experiences, and others fail to exclude the possibly direct effects of stress procedures on the fetus. Consequently no experiment yet reported has conclusively implicated gestational stress mediated by the mother during pregnancy as the cause of the effects on offspring behavior.

To unravel the roles of the two organisms involved—the mother and the fetus—requires procedures enabling any effects detected to be attributed conclusively to the prenatal period. There is evidence that the experiences of mothers prior to the birth of their litters can affect the subsequent behavior of their offspring (*4*) and, in order to avoid confounding prenatal and postnatal maternal influences (*3*) in such studies, offspring must be reared not by their natural mothers, but by foster-mothers.

In my experiment females of the 23rd generation of selection of the Maudsley reactive (MR) and nonreactive (MNR) strains, bred for high and low emotional defecation, respectively (*5*), were subjected to one of three prenatal treatments:

1) Premating avoidance training plus gestational stress: training (14 days) to avoid shock (0.3 ma) on presentation of a conditioned stimulus (illumination change) in a shuttle box; they were mated and given 18 days of further trials in the shuttle box with no shock presented, and the avoidance response was physically blocked on 16 of the 24 daily trials.

2) Premating avoidance training only: 14 days of shuttle box training, mating, no further disturbance.

3) Controls: no disturbance other than mating.

Females were mated in all the four possible combinations of the two strains (a 2 by 2 diallel cross) to enable the contributions of maternal and fetal genotypes, and their interaction with prenatal stress, to be evaluated separately. All litters born to these females were fostered shortly after birth (from 2 to 34 hours, mean 8.3) to untreated females of the MNR strain in order to equate postnatal environmental variables for all offspring. The offspring were weaned at 21 days, tagged by punching metal identification clips in their ears at 50 days, and otherwise left undisturbed until tested at approximately 100 days of age.

At this stage 96 offspring (two males and two females randomly drawn from each of 24 litters), equally divided among the four offspring genotypes of the diallel table (the two pure-bred strains and their reciprocal F_1's) and the three imposed prenatal treatments, were tested in the standardized open-field test of emotionality (four daily 2-minute trials) (*5*) and on an avoidance-conditioning task (one 50-trial session; unconditioned stimulus, shock of 0.25 ma; conditioned stimulus, a buzzer), half the offspring being tested in the order stated and half in the reverse.

Two of the several possible statistical analyses of diallel crosses (*6*) were applied to these data (Table 1). Main effects and interactions based on comparisons between litters in the factorial analysis were tested against errors between litters; if between-litter error was not significantly different from within-litter error, the two were pooled to provide an overall error variance.

The significant interactions between the paternal-strain factor and prenatal-treatment factor and that between genotype factor and prenatal-treatment factor in analyses of open-field ambulation

Table 1. Analysis of variance of diallel cross of open-field ambulation scores. M, the effects of maternal strain; P, of paternal strain; T, of prenatal treatment; G, of offspring genotype, and R, reciprocal (F_1) differences. Higher order interactions and sex and order effects are not included in the factorial analysis; of these only sex ($F = 49.90$, $P < .005$) was significant. d.f., degrees of freedom; M.S., mean square.

Source	d.f.	M.S.	F
Factorial analysis			
M	1	28,946.76	17.44*
P	1	810.84	
T	2	13.51	
M × P	1	4,746.09	2.86
M × T	2	1,009.39	
P × T	2	6,709.09	4.04†
M × P × T	2	721.59	
Residual	83	1,659.79	
"Hayman" analysis			
G	1	19,723.52	12.15*
R	1	10,034.09	6.18†
T	2	13.51	
G × R	1	4,746.08	2.92
G × T	2	6,420.58	3.96†
R × T	2	1,317.89	
G × R × T	2	712.11	
Residual	71	1,622.96	

* $P < .005$. † $P = .025$ to .01.

scores shown in Table 1 indicate that fetuses of different genotypes react differently to prenatal stress: offspring of MNR fathers showed reduced activity, those of MR fathers an increase. It was the premating training which was effective, since the scores of offspring of females stressed during gestation did not differ significantly from those of untreated controls. Thus training mothers on an avoidance-conditioning task prior to mating had bidirectional effects on offspring ambulation similar to those previously reported to result from stress during pregnancy (*1, 3*). The usual MR-MNR strain difference in ambulation is detected by the significant maternal strain effect (M in Table 1), and there is also a nonheritable maternal effect (R), with reciprocal cross (F_1) offspring significantly resembling the maternal strain. In addition, females ambulated significantly more than males (Table 1, footnote); test-order did not affect ambulation.

The open-field defecation scores of offspring were not affected by the prenatal treatments. The expected strain and sex differences were found, with MR offspring defecating significantly more than MNR offspring ($F = 84.84$, $P < .005$) and males more than females ($F = 6.21$, $P < .025$). Prior testing in the avoidance-conditioning apparatus resulted in significantly more open-field defecation, particularly amongst the F_1 males (interaction of genotype, sex, and test-order: $F = 5.91$, $P < .025$).

Results for avoidance conditioning showed that offspring of mothers subjected to gestational stress scored significantly more avoidances ($F = 3.87$, $P < .025$) with shorter response latencies ($F = 3.45$, $P < .05$) than those of untreated controls or offspring of mothers given premating training only; these last two groups did not differ significantly on either measure. Offspring genotype interacted with both prenatal treatment and postnatal (test-order) environmental variables; offspring of MNR fathers conditioned better than those of MR fathers in respect to both number of avoidances ($F = 4.15$, $P < .05$) and latencies ($F = 5.29$, $P < .025$).

Growth was also observed: untreated control offspring gained significantly more weight between birth and weaning than offspring of either of the prenatally treated groups ($F = 21.73$, $P < .05$). Changes in the open-field ambulation scores of the MNR foster-mothers that were tested before the experiment and again after the litters were weaned indicate (*7*) that those rearing untreated control offspring were affected by their litters differently from those rearing litters of the other two groups: the foster-mothers rearing control litters showed a marked decline in their ambulation from the preexperimental to postweaning testing ($F = 4.13$, $P < .05$, whereas those rearing litters whose biological mother had been subjected to premating or gestational stress showed virtually no change. The result of this subsidiary experiment suggests that the weight differences found here among the offspring are attributable to differences in their suckling behavior and resulting from the prenatal treatments given to their biological mothers; this interpretation is supported by the eventual disappearance of the weight difference after weaning. It also highlights the intricacy of the relation between offspring and mother or foster-mother which is further demonstrated in another subsidiary experiment in which the stressed mothers discarded from the experiment at the birth of their litters were themselves used to rear a standard group of MNR foster-pups—actually the offspring of the present MNR foster-mothers. The results (*8*) showed that the adult avoidance conditioning, but not the emotionality, of these untreated offspring was affected. Subjects reared by foster-mothers subjected to premating stress are significantly inferior in respect to both number of avoidances and their latency to those reared by control females; offspring reared by gestationally stressed rats were intermediate. Such findings from the two subsidiary experiments reinforce the appropriateness of the technique employed here of fostering all subjects to nonstressed mothers.

This experiment indicates conclusively that treatments applied to rats prior to the birth of their offspring and which affect the behavior of those offspring can be mediated prenatally by the mother; such effects occurred under conditions where postnatal maternal or other environmental influences and direct effects of the maternal treatments on the fetuses have been excluded. The direction of the effects which occur is dependent on the genotype of the offspring, with progeny of fathers of a high-ambulating strain having their activity reduced and those of fathers of a low-ambulating strain having theirs increased by stress applied to their mothers prior to mating. It is suggested that the occurrence of effects depends on the nature or timing of the treatment, since open-field ambulation was affected by premating stress only, whereas avoidance conditioning was affected by gestational stress only; the important difference between stress before and after mating may be the period of development at which the fetus is affected.

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