BEHAVIORAL BIOLOGY, 13, 113-119 (1975), Abstract No. 4158

BRIEF REPORT

Placentophagia in Nonpregnant Nulliparous Mice: A Genetic Investigation[1]

MARK B. KRISTAL and BASIL E. ELEFTHERIOU

Department of Psychology,
State University of New York at Buffalo,
Buffalo, New York 14226

and

The Jackson Laboratory,
Bar Harbor, Maine 04609

The genetic influence on the response of nonpregnant nulliparous mice to foster placenta was investigated. Two highly inbred strains (BALB/cBy and C57BL/6By), their F_1 hybrids, a backcross generation, and seven recombinant-inbred strains derived from the F_2 generation were tested. It was concluded that there is a genetic component to the response of female mice to placenta in the absence of previous experience, and that more than one, but possibly as few as two loci are involved. Alternative explanations of average dominance for placentophagia and for no placentophagia (by the promotion of competing responses) were considered.

Placentophagia, the eating of the "afterbirth," is manifested by females of most placental mammalian species during parturition. Despite the fact that in these species placentophagia is an integral component of perinatal maternal behavior, little is understood of the causes and consequences of the phenomenon. Parturition, or at least pregnancy, would seem to be the critical factor in the initiation of placentophagia. Recent studies have indicated, however, that parturition serves more as the mechanism for the presentation of placenta than as the causal factor in the initiation of placentophagia, since placentophagia was observed in a proportion of nonpregnant female rodents when placenta was made available (Kristal, 1973; Kristal and Williams, 1973; Sachs, 1969). Furthermore, the behavior is strongly dependent on experience,

[1]This research was supported, in part, by Training Grant MH-12126, from the National Institute of Mental Health to the Jackson Laboratory, where the animals in the present study were tested (The Jackson Laboratory is fully accredited by the American Association for Accreditation of Laboratory Animal Care), in part by funds from the Department of Psychology, State University of New York at Buffalo, and in part by Grant HD-05860 from the Institute of Child Health and Development.

since in both rats and mice, the proportion of a group of nonpregnant females manifesting placentophagia increased significantly with parturitional experience (Kristal, 1973; Kristal and Williams, 1973). Kristal (1973) demonstrated that previous parturitional experience, and not the pregnant condition was the key factor in predicting the occurrence of placentophagia during aphagia produced by lateral hypothalamic lesions.

Kristal and Williams (1973) investigated the influence of genotype (strain) and of reproductive condition on placentophagia in nonpregnant mice and found that strain differences contributed significantly to the attitude of mice toward placenta under all three reproductive conditions tested (virgin, primiparous without nursing, primiparous with nursing). Before investigating the mechanisms and parameters of the acquisition of experience relevant to placentophagia, the significance of placentophagia to maternal behavior, and the shift in neural control of placentophagia with the acquisition of experience, it seemed necessary to further investigate the factors influencing the base-level response to placenta (that manifested by virgin females). Since strain-comparison studies provide only an indication of genetic differences, it was decided to concretize the indication by conducting a genetic analysis of the strain differences observed by Kristal and Williams in nonpregnant nulliparous mice.

The subjects used in the present study were 16 virgin females of each of two highly inbred strains (C57BL/6By and BALB/cBy), 16 virgin females of each of the reciprocal F_1 hybrids of these two strains ($CB6F_1$ and $B6CF_1$), and 16 virgin females of each of seven recombinant-inbred (RI) strains derived from the F_2 generation of a cross between the C57BL/6By and BALB/cBy inbred strains (CXBD, CXBE, CXBG, CXBH, CXBI, CXBJ, CXBK). The RI-strain battery, derived in this manner from a cross between two highly inbred strains, and maintained independently on a schedule of strict inbreeding (brother X sister), can be considered to be a replicable recombinant population (Bailey, 1971). The strains of the RI-strain battery comprise only BALB/c and C57BL/6 genetic material, but the RI-strains differ from one another in the chance recombinations that have formed and which are fixed during the inbreeding process as the strains approach full homozygosity. Testing of all 11 strains of the battery (two progenitors, two F_1 hybrids, and seven RI-strains) plus certain crosses and backcrosses yields a strain distribution pattern from which information on the number of loci involved, the existence of dominance, the direction of dominance, maternal postnatal influences, and epistasis can be gleaned. If the strain distribution pattern of the seven RI-strains (the distribution of the progenitor alleles across those seven lines) matches a cataloged strain distribution pattern for a known gene, information regarding the locus of the gene or genes in question can be obtained. Finally, as a cross check for locus, many lines are available that are congenic for segments of chromosomes containing known genes whose strain

distribution patterns were cataloged. Testing of the appropriate congenic line in the experimental situation would verify the locus of the gene in question if the congenic line behaves like animals of the substituted genotype rather than like animals of the background genotype (Klein, 1973). Although the RI-strain technique is relatively new, several studies employing this particular RI-strain battery already exist in the literature (e.g., Eleftheriou and Bailey, 1972; Oliverio, Eleftheriou, and Bailey, 1973; Eleftheriou and Kristal, 1974).

All females were tested at 60-70 days of age. Each female was presented with one to three placentas, removed surgically from either a BALB/cBy or a C57BL/6By donor on the last full day of pregnancy. After removal from the donor, the placentas were frozen with a few drops of normal saline, and thawed immediately prior to testing. Body-temperature placentas were presented in small, untippable glass dishes for 15 min to females in individual, clean, plastic cages. All mice were housed on a 7:00 AM to 7:00 PM daylight cycle. Two hours prior to testing, food was removed from the cages of the test mice, and the cages were placed in the testing room. Fifteen minutes prior to testing, each female was placed in her test cage. The behavior of each female toward placenta was observed during the 15-min test. The presence or absence of placentophagia was scored; unless the placenta was picked up and some part of it was actually eaten, a negative score was given. The test was easy to score in that the behaviors of the eaters and noneaters were clearly different.

The results of the exposure of virgin females of the RI-strain battery to placenta are presented in Table 1.

TABLE 1

Incidence of Placentophagia in Nonpregnant Nulliparous Female Mice

Group tested	Placenta used in test		Total
	BALB/cBy	C57BL/6By	
BALB/cBy	2/8	2/8	4/16
C57BL/6By	0/8	0/8	0/16
$B6CF_1$	0/8	0/8	0/16
$CB6F_1$	0/8	0/8	0/16
CXBD	1/8	1/8	2/16
CXBE	0/8	0/8	0/16
CXBG	6/8	1/8	7/16
CXBH	0/8	0/8	0/16
CXBI	0/8	0/8	0/16
CXBJ	0/8	0/8	0/16
CXBK	2/8	1/8	3/16
$B6CF_1$ × BALB/cBy	11/24	8/24	19/48
(CXBH × CXBG) × CXBG	16/31	23/31	39/62
B6.C-*H-25*^{c}	0/3	0/4	0/7

The progenitor strains BALB/cBy and C57BL/6By show an incidence of placentophagia of 25% and 0%, respectively. These values are in accord with values found previously for the two strains (Kristal and Williams, 1973). The difference between the progenitor strains in response to placenta has a probability of occurring by chance of about .05 ($P = 0.0506$), as determined by the Fisher exact-probability test.

The absence of placentophagia in the F_1 hybrids ($CB6F_1$ and $B6CF_1$) indicates that if the progenitor strain difference is genetically determined, C57BL/6 alleles of the gene or genes of influence are dominant. In addition, the absence of a difference between the reciprocal F_1 hybrids indicates the absence of an effect attributable to the genotype of the mother. Testing of a backcross generation consisting of the offspring of $B6CF_1$ females backcrossed to BALB/cBy males exhibited an incidence of placentophagia of 39.5% (19/48).

Of the seven strains constituting the RI-strain battery, placentophagia was seen in only three: CXBD, CXBG, and CXBK (see Table 1). The incidence of placentop agia in the CXBD and CXBK strains was lower than that of the BALB/c progenitor strain, but 43.7% of the females of the CXBG strain (7/16) manifested placentophagia. To obtain information about the result of mating the CXBG strain (high incidence of placentophagia) with a strain which did not manifest placentophagia, the CXBH strain, female offspring of a cross between CXBH females and CXBG males were backcrossed to CXBG males. The incidence of placentophagia in this backcross was 62.9% (see Table 1). The strain distribution pattern did not match any of the cataloged strain distribution patterns for known loci across this same RI-strain battery. The inability to match strain distribution patterns precluded verification of effective loci by the use of the congenic lines (Klein, 1973). It should be noted that one congenic line was tested. The B6.C-*H-25*^{c} congenic line (C57BL/6 mice with a substituted piece of Chromosome 9 containing the BALB/c allele of the *H-25* histocompatability locus) bears a strain distribution pattern almost identical to that produced by testing the RI-strain battery for placentophagia. The difference lies in the fact that the score for the CXBG RI-strain is considerably greater than that of the BALB/c progenitor. To be identical to the distribution of alleles found in the B6.C-*H-25*^{c} congenic line the CXBG RI-strain would have to have manifested an incidence of placentophagia not different from the BALB/c progenitor. The similarity between the strain distribution pattern of B6.C-*H-25*^{c} and that of the RI-strain battery in the present study seemed great enough to warrant testing of B6.C-*H-25*^{c}, despite the fact that negative results were expected. Seven virgin B6.C-*H-25*^{c} females (obtained from the colony of D.W. Bailey) were tested for placentophagia; none manifested the behavior (see Table 1).

A single-locus explanation was rejected on the basis of the incidence of placentophagia in the progenitor strains and the high incidence of placentophagia in the backcross generation and in the CXBG RI-strain. Models consiting of as few as two loci can be invoked. If two loci with average dominance for placentophagia is the case, the alleles of the loci in question are apparently not isodirectionally distributed, in that the BALB/cBy progenitor does not possess all the alleles required to produce placentophagia in 100% of the females. Perhaps both progenitor genotypes harbor "increasors." For some reason, possibly epistasis, the BALB/cBy progenitor genotype (A/A, +/+) results in placentophagia in 25% of the females, the C57BL/6By progenitor genotype (+/+, B/B) results in placentophagia in 0% of the females. The F_1 hybrid genotype (A/+, B/+) also results in the promotion of placentophagia in 0% of the females. The one-fourth of the B6CF_1 × BALB/c backcross generation comprising the BALB/c progenitor genotype, and the one-fourth of the backcross generation comprising the double-heterozygous genotype (F_1) together can only account for the promotion of placentophagia in 6.25% of the females of the backcross generation. The remaining two genotypes of the backcross generation (A/+, +/+ and A/A, B/+) must together promote placentophagia in about 66.6% of the backcross females of those genotypes. The high incidence of placentophagia in the first backcross and in the (CXBH × CXBG) × CXBG RI-strain backcross (62.9%) can be considered as further evidence of the presence of hidden increasors. Pursuing the notion of hidden increasors further, we might consider that the two recombinant genotypes constituting 50% of the B6CF_1 × BALB/c backcross generation (A/+, +/+ and A/A, B/+) promote placentophagia to a different extent. The addition of one B allele to the BALB/c genotype, or the removal of one A allele from the BALB/c genotype could conceivably produce incidences of placentophagia of approximately 50% and 75%. The resulting incidence of placentophagia in the entire backcross population would then be predicted to be 0% of ¼ + 25% of ¼ + 50% of ¼ + 75% of ¼, or 37.5%. The observed value for placentophagia in the backcross generation was 39.6% (19/48). The expected value of 37.5% and the observed value of 39.6% are not significantly different and in fact, in a sample of 48 mice represents a difference of only one mouse.

The recombinant-inbred strains provided little decisive information beyond that obtained from the progenitor strains, F_1 hybrid and backcross generations, owing to our inability to match strain distribution patterns. The incidence of placentophagia in the CXBG RI-strain (43.75%) indicates that a genotype producing approximately 50% placentophagia, such as that hypothesized to exist in the backcross generation, is a distinct possibility. But neither that observation, nor the incidence of placentophagia in the

(CXBH × CXBG) × CXBG backcross eliminates the possibility that the progenitor strains both harbor increasors for placentophagia. The explanation of the 37.1% incidence of placentophagia in the RI-strain backcross is difficult to explain without further information about why the genotype of the CXBH females results in 0% placentophagia.

The CXBG RI-strain was the only group tested that exhibited an unequal response to the two types of placenta used. This unequal and unreplicated response, since it occurred in only one of the 14 groups tested, is unexplainable. Although the response of the (CXBH × CXBG) × CXBG females might be interpreted as being unequal, the difference in response of this group to the two types of placenta is proportionally small, and is in a direction opposite to that observed in the CXBG group.

It is difficult to conceptualize the motivational mechanism underlying placentophagia in nulliparous females, considering that virtually all female mice eat placenta at parturition. Why would only some of the nulliparous females with a particular genotype manifest placentophagia? The answer may lie in the behavior of the nulliparous females that do not eat placenta. Kristal and Williams (1973) reported that among virgin mice not eating placenta, many backed into the farthest corner of the cage from the dish of placenta and emitted tail-rattling. The same phenomenon was seen, but not quantified, in the present study. Perhaps avoidance (aversion, fear, neophobia) of certain types of stimuli in the absence of previous experience is actually the direct result of average dominance of the alleles in question. Placentophagia in virgins could then be considered to be the consequence of the absence of some of the alleles responsible for avoidance of the substance, or inhibition of placentophagia. At parturition, the hormonal milieu and sequence of behavioral events may then override the behavioral responses competing with ingestion of this particular novel substance, ultimately resulting in the disinhibition of placentophagia. Subsequent to parturition, the genotype of the female would then determine the facility with which placenta is removed from the conceptual category of novel substances as evidenced by the interaction of strain and parturitional experience observed in the study by Kristal and Williams.

Although the analysis of the genetic influence on placentophagia in nonpregnant nulliparous female mice has been neither exhaustive nor conclusive, certain general statements can be made. We feel that it is safe to conclude from the data that there is a genetic component to the response of female mice to placenta in the absence of previous experience; that the genetic component is complex and certainly involves more than a single locus, but that two-loci models are feasible; that alternative genetic models considering that the result of average dominance of the alleles either can produce placentophagia, or can produce no placentophagia by promoting a competing response, are both conceivable.

REFERENCES

Bailey, D. W. (1971). Recombinant-inbred strains. *Transplantation* **11**, 325-327.

Eleftheriou, B. E., and Bailey, D. W. (1972). Genetic analysis of plasma corticosterone levels in two inbred strains of mice. *J. Endocrinol.* **55**, 415-420.

Eleftheriou, B. E., and Kristal, M. B. (1974). A gene controlling bell- and photically-induced ovulation in mice. *J. Reprod. Fert.* **38**, 41-47.

Klein, J. (1973). List of congenic lines of mice. *Transplantation* **15**, 137-155.

Kristal, M. B. (1973). Effects of lateral hypothalamic lesions on placentophagia in virgin, primiparous, and multiparous rats. *J. Comp. Physiol. Psychol.* **84**, 53-62.

Kristal, M. B., and Williams, C. L. (1973). The effect of strain, reproductive condition, and strain of placenta donor on placentophagia in nonpregnant mice. *Physiol. Psychol.* **1**, 354-356.

Oliverio, A., Eleftheriou, B. E., and Bailey, D. W. (1973). Exploratory activity: Genetic analysis of its modification by scopolamine and amphetamine. *Physiol. Behav.* **10**, 893-899.

Sachs, B. D. (1969). Behavior of maternal rats in the perinatal period. *Amer. Zool.* **9**, 1068.