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Studies on Wild House Mice. VII. Prenatal Maternal Environment and Aggression

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The effect of the maternal environment on intermale aggression was studied by means of embryo transfer of genetically selected aggressive (SAL) and nonaggressive wild house mice (LAL), and their reciprocal F_1's, to standard (NMRI) females. No effect was found on the attack latency scores (ALS), i.e., aggression: all genotypes born and raised under natural conditions showed an ALS similar that of genotypes born and raised by NMRI females. Since previous studies on wild house mice failed to demonstrate postnatal effects on aggression, and the present results indicate the absence of prenatal maternal environmental effects on aggression, the primacy of genetic over maternal variance in the development of adult intermale aggression in wild house mice is indicated.

KEY WORDS: Aggression; maternal effects; wild house mice; Y chromosome; embryo transfer.

INTRODUCTION

Genotypic variation is an important factor underlying individual differences in aggression in rodents. One of the means to study the genetic correlates of behavior is artificial selection. In our laboratory we have successfully selected male wild house mice (*Mus musculus domesticus*) for attack latency (van Oortmerssen and Bakker, 1981). Bidirectional selection has resulted in an aggressive line, characterized by a short attack latency (SAL), and a nonaggressive line, having a long attack latency (LAL).

However, apart from genotypic variation, differences in attack latency may be due to several other sources of variation. One of these sources is genomic or gametic imprinting; this phenomenon describes those parental-dependent traits in which both the male and the female allele are present but function unequally in the embryo (for a recent review, see Barlow, 1995). Another source is maternal factors. As stated by Roubertoux *et al.* (1990), maternal aspects can be either genetic (X chromosomal, mitochondrial DNA) or environmental (cytoplasmic, prenatal, or uterine and postnatal). The present paper concentrates on the prenatal maternal environmental component of aggression.

Ressler (1962) was probably one of the first researchers to claim that among experimental animals variations in the parental, i.e., maternal, environment must be identified before evaluating genetic effects. Pertaining to aggression, the NZB and CBA/H inbred mouse lines have been studied most intensively with respect to maternal environmental effects on this behavioral trait. The results indicate an absence of both prenatal and postnatal maternal effects on offensive attack behavior (Roubertoux and Carlier, 1988). However, the absence of this effect is restricted to the parental strains, because agonistic differences between re-

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ciprocal F_1's depend on the maternal environment, albeit in interaction with genetic, i.e., Y chromosomal, factors (Carlier *et al.*, 1991; Roubertoux *et al.*, 1994). For an enumeration of both prenatal and postnatal maternal environmental effects of agonistic behavior (offense) in male mice, the reader is referred to Maxson (1992).

Regarding our selection lines, previous studies have demonstrated the nonexistence of postnatal maternal effects on aggression scores in both SAL and LAL mice (van Zegeren, 1980; van Oortmerssen *et al.*, 1985) and reciprocal crosses between these lines (Sluyter *et al.*, 1995b). Focusing on prenatal effects, we have standardized here the general maternal environment (the pre- and postnatal environment) of both selection lines and their reciprocal F_1's.

There are two ways to do this: the ovarian graft method and embryo transfer (ET). For a detailed description of both techniques, their advantages and disadvantages, the reader is referred to Carlier *et al.* (1992). Contrary to ovary grafting, ET does not require histocompatibility and thus allows for a transfer between different strains. Embryos may be transferred to recipients via the oviduct or the uterus using surgical transfer or via the cervix into the uterus using nonsurgical transfer. We have chosen the latter, less stressful method. For recipients we have selected NMRI females; they are outbred and characterized by relatively low aggressiveness toward newborns as well as by substantial milk production (Pierre Roubertoux, personal communication).

MATERIALS AND METHODS

Mice

All lines bidirectionally selected for attack latency originated from a colony of wild house mice (*Mus musculus domesticus*) maintained in our laboratory since 1971. The mice were housed in Plexiglas cages (17 × 11 × 13 cm) in a room with an artificial 12:12 LD cycle (dark from 1230). Food (standard laboratory chow; Hope farms AM2) and water were available ad libitum. At weaning age (3–4 weeks), the litters were separated from their parents. At the age of sexual maturity (6–8 weeks), the animals were paired male–female. At the age of 14 weeks the males were tested for aggression.

Aggression Test

The aggression test has been extensively described by van Oortmerssen and Bakker (1981). The test was performed at the Department of Animal Physiology, Haren, The Netherlands. The time it takes for a given animal to attack a standard opponent was measured on 3 consecutive days (days 3 to 5), determined between 1300 and 1500, during the dark phase (lighting: 15-W bulbs). Before the actual testing the animal had the opportunity to explore the border area of the test cage for 1 h on 2 days (days 1–2). Such a border area was created since most agonistic encounters between mice occur in nature at the border of the male's territory (Crowcroft, 1966). The mean time of three tests was its attack latency score (ALS). Attack latency is a reliable index of aggression, since there is a significantly negative correlation between attack latency and the number of attacks and accumulated attacking time, including chasing, biting, and fighting (Catlett, 1961; van Zegeren, 1980). Each experimental male was confronted with a standard, gonadally intact male albino mouse (MAS-GRO). Standard opponents should elicit offensive behaviors from the experimental animal but not initiate offensive behaviors themselves (Denenberg *et al.*, 1973). The opponents used in this study had been attacked and had shown defensive behavior in previous tests. The test was terminated immediately after the experimental animal attacked the opponent or, in the absence of any attack, after 10 min. Therefore, for those males that did not fight on 3 consecutive days, the ALS was set at 600 s.

Embryo Transfer

The nonsurgical method used is slightly modified from van der Hoeven *et al.* (1991). The females (SAL and LAL) used as embryo donors were 3–5 months of age. Superovulation was achieved by an intraperitoneal injection of 2.5 IU PMSG at 1130 during dioestrus, as observed by vaginal smears (Thung *et al.*, 1956), followed by a second injection with 2.5 IU hCG 48 h later. Females were caged with, but physically separated from, males after the first injection. Immediately after the second injection the females were exposed to the males. The females were checked for copulation plugs the morning after the second injection. Embryos were recovered on the morning of Day 4 (day

Table I. Mean Embryo Production, Blastocysts (%), Number of Embryos Transferred, Successful Embryo Transfers (%), Young Born, Survival Rate (%) for All Recipients and for Pregnant Recipients Only in Both Selection Lines (SAL, LAL), the Albino Variant of the SAL Type (SAL-PE), the Mixed Group, and Both Reciprocal F_1's (LAL.SALF1 and SAL.LALF1): Mean ± SE (Numbers Are Given in Parentheses)

Genotype	Embryo production (No.)	Blastocysts (%)	Successful transfers (%)	Embryos transferred (No.)	Young born (No.)	Survival rate (%)	Survival rate, pregnants only (%)
SAL	11.4 ± 1.5 (9)	98 ± 2	71	9.9 ± 0.2 (7)	5.2 ± 1.4	50 ± 14	70 ± 8 (5)
LAL	9.9 ± 1.1 (24)	63 ± 8	40	8.9 ± 0.3 (15)	2.5 ± 0.9	27.9 ± 10	70 ± 8 (6)
SAL-PE	7.6 ± 0.8 (14)	74 ± 9	60	7.2 ± 0.2 (5)	2.6 ± 1.2	36.1 ± 17	60 ± 14 (3)
Mixed (SAL-PE+LAL)	See SALPE and LAL	See SALPE and LAL	73	10 ± 0 (11)	4.8 ± 1.1	48.2 ± 11	66 ± 8 (8)
LAL.SALF1	11.0 ± 1.4 (8)	75 ± 11	75	8.9 ± 0.6	4.9 ± 1.1	49.6 ± 11.6	66 ± 6 (6)
SAL.LALF1	9.6 ± 1.3 (7)	97 ± 3	67	10.3 ± 0.2 (6)	6.2 ± 2.0	62 ± 20	93 ± 5 (4)

of plug is Day 1), classified as morulae and blastocysts, and pooled per morphology group. NMRI/HsdWin females were used as recipients. Recipients always had natural cycles and were made pseudopregnant by mating with either vasectomized or genetically sterile males. For a detailed description of this technique, see van der Hoeven *et al.* (1991).

Experimental Procedure

For embryo transfer six groups were created: (1) SAL, (2) LAL, (3) a pink-eyed variant of the SAL type (abbreviated SAL-PE), (4) a mix between SAL-PE and LAL, and (5, 6) the two reciprocal F_1's. The latter two groups are denoted LAL.SALF1 and SAL.LALF1 (the genotype of the dam being listed first, followed by that of the sire). Group 4 was formed to check for the effect of genotypical variation in the outbred recipients on the ALS. Equal numbers of SAL-PE and LAL embryos were transferred. The gray coat color and the pink eyes of SAL-PE were used as markers to discriminate between SAL and LAL. The SAL-PE (sub-)strain originated from a spontaneous mutation 5 years ago, which has since occurred irregularly. Newborn SAL-PE mutants have been introduced to the substrain to prevent inbreeding. As we never actually tested for the presence of the pink-eyed dilute gene, we use a different abbreviation for this SAL-mutant (SAL-PE instead of SAL-p). As with the p-gene mutants in other strains, the mode of the PE inheritance is recessive. SAL-PE males show ALS values similar to those of the original SAL males (van Oortmerssen, unpublished research).

SAL, LAL, and their reciprocal F_1's, which were born and raised by their natural mothers, were used as controls. Body weights were determined on day 1 of the aggression test.

The number of embryos produced and the percentage of blastocysts were analyzed using a classical one-way ANOVA with five levels (embryo groups 1–3, 5, 6). All other embryo recovery data were analyzed using a one-way ANOVA with six levels (all six embryo groups). To check for the effect of genotypical variation in the outbred recipients on the ALS, a two-way ANOVA was applied, with one factor being the genotype (with two levels: SAL and LAL) and the other factor being the different uterine environment (with two levels: mixed and "nonmixed"). If no effects were detected, the data were pooled per genotype.

For ALS and body weights a two-way ANOVA was used, with one factor being the different genotypes (with four levels: SAL, LAL, and both reciprocal F_1's) and the other factor being the different maternal environment (with two levels: natural and NMRI mothers).

RESULTS

Table I shows the results of the embryo recovery. A one-way ANOVA with genotype as main

factor shows a significant value only for percentage of blastocysts [$F(4,58) = 3.2$, $p < .05$], with SAL mothers producing higher percentages of blastocysts than LAL ones.

SAL males that shared their general (NMRI) maternal environment with LAL males (the "mixed" group) did not differ in ALS to those SAL animals that shared their general (NMRI) maternal environment with SAL ones. Conversely, the same is true for LAL males. Consequently, these data were pooled per genotype. The ALS of both control and embryo transferred genotypes are shown in Fig. 1. ALS was not influenced by the general maternal environment, whereas it was affected by the origin of genotype [$F(7,170) = 74.1$, $p < .001$]. No interaction effects were found.

Table II shows the mean body weight of both control and embryo transferred genotypes. No significant effect of genotype on body weight was found. However, the general maternal environment did influence body weight, with genotypes born and raised under natural conditions showing lower body weights than those born and raised by NMRI mothers [$F(7,160) = 43.6$, $p < .001$]. The interaction almost reached significance [$F(7,160) = 2.4$, $p = .07$].

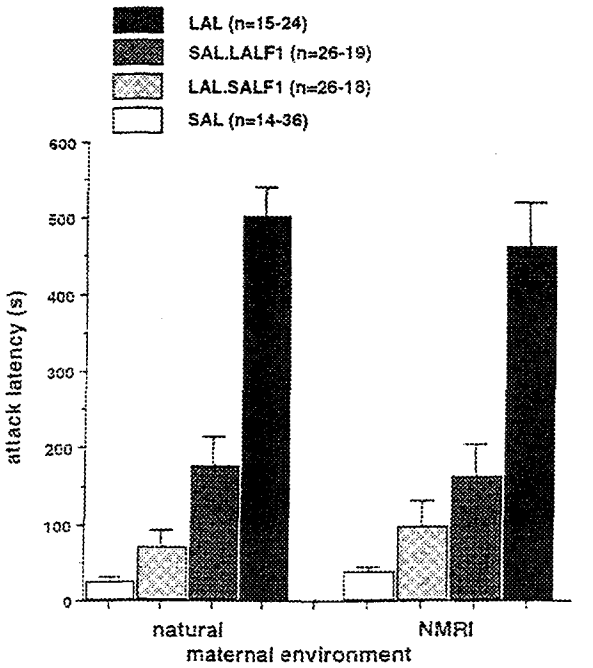

Fig. 1. Mean attack latency scores (ALS) of both selection lines (SAL and LAL) and their reciprocal F_1's (SAL.LALF1 and LAL.SALF1) when born and raised by their natural mother and when born and raised by a NMRI female. Numbers of animals are in parentheses [the first number corresponds to the number of controls (natural); the second, with the number of animals transferred to NMRI females].

DISCUSSION

This study shows that standardizing the general maternal (pre- and postnatal) environment by means of embryo transfer did not affect attack latency scores (ALS) in artificially selected male wild house mice. When born and reared by standard NMRI recipients, aggressive SAL and nonaggressive LAL males and their reciprocal F_1's displayed an ALS to that of controls born and reared by their natural mothers. The origin of the genotype, though, undisputedly affected aggression, with SAL having a shorter, LAL a longer, and reciprocal F_1's an intermediate ALS. This distinct, genotypically dependent, aggression pattern corresponded to previous findings (Sluyter *et al.*, 1994; van Oortmerssen *et al.*, 1992).

As mentioned in the Introduction, no postnatal effects on aggression have been found in either SAL or LAL (van Zegeren, 1980; van Oortmerssen *et al.*, 1985) or their reciprocal F_1's (Sluyter *et al.*, 1995b). The findings in the present study, in addition, indicate the absence of prenatal maternal environmental effects on aggression in wild house

Table II. Mean Body Weight of Both Selection Lines (SAL and LAL) and Their Reciprocal F_1's (SAL.LALF1 and LAL.SALF1) When Born and Raised by Their Natural Mother and When Born and Raised by a NMRI Female: Mean ± SE (Numbers are Given in Parentheses)

Genotype	Natural	NMRI
SAL	20.3 ± 0.4 (13)	21.5 ± 0.3 (34)
LAL	19.8 ± 0.6 (15)	22.0 ± 0.2 (22)
SAL.LALF1	20.2 ± 0.3 (23)	21.2 ± 0.3 (18)
LAL.SALF1	19.0 ± 0.4 (26)	21.8 ± 0.3 (17)

mice. Some caution should be exercised in this regard, however. First, by using a third strain as recipient instead of a cross-transfer design (with SAL females as recipients for LAL and LAL.SALF1 and LAL as recipients for SAL and SAL.LALF1), we cannot exclude both maternal environmental effects of SAL and LAL on aggression and possible transfer effects per se. The latter effects, corrected for litter size reduction, have not been reported in mice. However, in sheep, embryo-transferred ani-

mals tended to be heavier than controls (Walker *et al.*, 1992). If such a transfer effect also existed in the mice studied here, it did not apparently affect aggression scores, since the general increase in body weight after embryo transfer did not influence ALS. Second, both the environmental cytoplasmic effect and the influence of the biological mother before the time of transfer (morula or blastocyst stage) cannot be voided. Nevertheless, the genotypic diversity of the outbred NMRI females can be eliminated as a source of prenatal variation. Sharing completely identical pre- and postnatal environments, i.e., the same NMRI recipient, SAL and LAL males, showed an ALS similar to that of their "nonmixed" controls.

These data are in agreement with those on the previously mentioned NZB and CBA/H strains. Using the ovarian graft method, no prenatal effects were found in the parental strains (Roubertoux and Carlier, 1988), whereas the maternal effect on attack behavior in the reciprocal F_1's was likely to be postnatal (Carlier *et al.*, 1991). There are some small indications, though, that prenatal effects on intermale aggression may exist in mice, albeit few and not explicitly evaluated for uterine effects. Although not specifically tested for maternal, i.e., prenatal, effects, the findings on agonistic scores of reciprocal F_1's in a diallel cross design cannot exclude such effects (Hahn and Haber, 1982). Another study, using ovarian grafting, was clearer in its design for testing prenatal effects. Eleftheriou *et al.* (1974) demonstrated that the prenatal environment may differentially affect intermale aggression. The attack scores of the aggressive CXBG recombinant inbred strain were decreased if exposed during prenatal development to the uterine environment of $B6CF_1$, whereas the scores of the less aggressive CXBH males did not change. However, when crossfostered to CXBH (and not $B6CF_1$) females, the aggression scores of CXBG males were similar to those of the ovary-transplanted CXBG males, suggesting a postnatal effect.

Furthermore, we found a correlated selection response on cleavage rates. The fact that the genotypes which originate from an SAL mother (SAL and SAL.LALF1) show higher percentages of blastocysts in relation to morulae may demonstrate a relatively delayed development for those that come from an LAL mother. If this difference in rate of preimplantation development is genetic, it is likely to be maternal (X chromosomal, mitochondrial DNA) and not Y chromosomal, as has been reported for some laboratory strains (Burgoyne, 1993). However, other sources of variation, such as cytoplasmic factors and time of conception, may also be responsible for this difference.

The variation in preimplantation stage seems also not to be related to differences in aggression in wild house mice. Reciprocal F_1's with the LAL Y chromosome, which are less aggressive than their counterparts with the SAL Y chromosome, show higher percentages of blastocysts. This does not necessarily mean that differences in developmental rates per se are not important in the ontogeny of aggression. One must keep in mind that different prenatal developmental stages at the time of transfer may be nullified or even reversed by the time of birth. Consequently, the possibility exists that the reciprocal F_1's which develop faster during preimplantation mature less during the postimplantation period. Conversely, the slower-developing F_1's may mature faster during postimplantation. In this context, it is important to mention that both the fetal heredity and the maternal environment contribute significantly to the more advanced development of hybrid (i.e., reciprocal F_1's) mice (Wahlsten and Wainwright, 1977). Genetic factors, possibly Y chromosomal, may reverse the developmental retardation of the preimplantation stage.

Summarizing, aggression scores in NMRI-born-and-reared SAL, LAL, and reciprocal F_1's mice were similar to those of their natural born and reared counterparts. These findings, in combination with the known absence of postnatal effects, illustrate the importance of genetic over maternal variance in the development of adult aggression patterns. Future experiments will show whether these findings are limited to aggression only or may be expanded to related behavioral strategies such as those described by Sluyter *et al.* (1995a, 1996), Benus *et al.* (1991), and Bohus *et al.* (1987).

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