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The Effect of Fostering on the Genetic Expression of Locomotor Sensitivity to Alcohol

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Steps were taken to eradicate endemic mouse coronavirus from a colony that was part of a behavioral project characterizing the genetics of alcohol sensitivity. This behavioral study was conducted to determine whether changing the uterine or rearing environment (as is integral to common rederivation methods) would have a significant effect on the expression of the behavioral traits in question. Selected breeding pairs of the affected lines were divided into four treatment groups: 1) transfer of embryos to pseudopregnant B6D2F1 female mice, 2) fostering offspring to B6D2F1 dams, 3) fostering offspring to a different dam of the same line, and 4) offspring raised by the birth dam. Embryo transfers were successful only in one affected line. At approximately 50 days of age, the offspring were tested for locomotor behavior after intraperitoneal administration of ethanol or normal saline. There were no statistically significant effects of embryo transfer on the ethanol phenotype (ethanol-induced locomotor depression). Fostering significantly reduced the stimulant response to ethanol of only one mouse line selectively bred for high sensitivity to ethanol-induced stimulation, although the stimulant response of the fostered groups was still quite robust. Overall, the results of this study showed that eradication efforts involving fostering of offspring have a modest impact on the stimulant response to ethanol, but there were insufficient data to draw conclusions regarding the use of embryo transfer.

Eradication of mouse coronavirus (also known as mouse hepatitis virus; MHV) after a confirmed outbreak in an animal facility requires the consideration of many different factors. There are well-established methods for eradication of mouse coronavirus, including embryo transfer (4, 20, 27), cross-fostering (14, 17), Caesarian rederivation (16), cessation of breeding (30), and testing and culling of individual animals (14, 26, 29). When selecting an eradication method, the effect of the virus and the method of eradication on the research project must be considered. The complicating effects of mouse coronavirus on research are well documented (2, 6). The effects of the methods of eradication on the research are not as well defined; these effects are unique to the research project being performed. We were concerned that methods requiring exposure to a unique uterine or rearing environment, such as embryo transfer, cross-fostering, or Caesarian rederivation, might introduce an undesirable confounding variable into our research. A significant effect of rearing environment has been reported in many species (1, 3, 5, 10, 18, 28). Intrauterine environment and postnatal care have been shown to influence behavior in adulthood (7, 11), and genes and environment can interact to influence behavior (12, 21). We performed a study to examine the possible influence of embryo transfer and cross-fostering on the behavioral traits that we desired to preserve in a genetic animal model of increased and reduced sensitivity to the locomotor stimulant effects of ethanol.

Materials and Methods

Mice. The two lines of selectively bred mice, FAST and SLOW, are collectively referred to as the activity selection (ACS) lines. The selection strategy for these lines has been described in detail previously (9, 24). All lines originated from a heterogeneous stock (HS/lbg) that was the product of an eight-way cross of inbred strains chosen for their divergent genetic backgrounds. Mice from the heterogeneous stock were tested in circular locomotor activity monitors (LVE model PAC-001, Lehigh Valley, Pa.) in which locomotion was detected by interruption of photocell beams after administration of ethanol or saline on two consecutive days. The saline score was subtracted from the ethanol score to create a stimulation score. The 36 mice (18 female and 18 male) with the highest stimulation scores were chosen as breeders to establish two independent FAST-1 and FAST-2 lines. These replicate lines then were maintained as independent breeding populations throughout 37 generations of selective breeding for extreme sensitivity to the locomotor stimulant effects of ethanol. Similarly, 36 mice with the lowest stimulation scores were chosen as breeders to establish two independent SLOW-1 and SLOW-2 lines bred for 37 generations for insensitivity to the stimulant effects of the same dose of ethanol. The two independent replicates of each line are maintained to provide stronger data interpretation (8). Nine families of each line are maintained, and a rotational breeding scheme was used during selection to preserve heterogeneity at loci not relevant to the selection traits; thus, across all lines, there are 36 families, each with a unique numeric identifier. Identification of the genetic loci influencing the selection responses is currently under investigation. The ACS lines have been maintained under relaxed selection conditions since generation 37. Mice from first litters serve as breeders for the subsequent generation, and then the parents of those first litter offspring serve as production breeders for mice used in experiments. Mice used for this study were from generation $S_{37}G_{68}$ (where S_{xx} refers to the number of generations of selective breeding, and G_{xx} refers to the total number of generations that the lines have existed since selection began).

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The Oregon Health & Science University Transgenic Animal Core performed all embryo transfers using C57BL/6J by DBA/2J F1 (B6D2F1) mice as the recipients. B6D2F1 mice also were chosen as the recipient strain for the cross-fostering manipulation for consistency with the use of this strain by the Transgenic Animal Core. The B6D2F1 and ACS mice were approximately 5 weeks of age at time of superovulation and breeding.

All mice were housed in polycarbonate shoebox cages with filter tops (Thoren Caging Systems, Hazelton, Pa.) and corncob bedding (Bed-O'Cobs, Maumee, Ohio). Cages were changed at least once weekly in a laminar flow changing station (Lab Products, Seaford, Del.). The animal caretakers wore latex gloves while changing cages and sprayed their gloves with a 10% bleach solution between each cage. Soiled cages were sanitized in a mechanical cage washer with a final rinse temperature of 180°F (82°C). The rooms were kept on a 12:12-h light:dark cycle (lights on, 0600 h), and animals were provided rodent chow (LabDiet, St. Louis, Mo.) and tap water ad libitum. Temperature and humidity were maintained at 72°F (22°C) and at least 30%, respectively. The Portland VA Medical Center Institutional Animal Care and Use Committee approved all projects using these mice, in accordance with applicable federal regulations.

Indirect exposure sentinel mice were used to screen the colony for pathogens on a quarterly basis. At least two sentinel mice, 5-week old female mice of the ACS lines or ICR strain mice (Taconic, Germantown, N.Y.), were provided for every 100 cages of mice, and each room typically had at least eight sentinel mice. Sentinel mice had been exposed to pooled dirty bedding from colony cages for a minimum of 21 days. Dirty bedding was collected at cage change, pooled, and used to create at least 60% of the bedding provided to the sentinel animals. Serum samples collected from these sentinel mice by cardiac exsanguination under isoflurane anesthesia were submitted to the University of Missouri Research Animal Diagnostic Laboratory (St. Louis, Mo.) for serologic testing. Typically, two sentinels from each room were saved in case additional sequential testing was required. Internal and external parasite screens were performed in-house. At the time of this experimental manipulation, the ACS lines of mice were determined to be free of Sendai virus, mouse parvovirus, minute virus of mice, ectromelia virus, reovirus type 3, pneumonia virus of mice, murine adenovirus, *Mycoplasma pulmonis*, lymphocytic choriomeningitis virus, mouse rotavirus, mouse encephalomyelitis virus, polyoma virus, murine cytomegalovirus, and rodent pinworms and mites. However, the core and production breeding colonies of the ACS lines of mice consistently tested positive for mouse coronavirus for more than a decade by using indirect sentinels. The B6D2F1 mice were negative for all screened pathogens listed.

Reproductive manipulation. (i) Embryo transfer. Embryos were produced using published methods (22). In short, 5-week-old SLOW-1, SLOW-2, FAST-1, and FAST-2 donor dams were treated intraperitoneally with 5 to 10 IU pregnant mare serum gonadotropin followed by 5 to 10 IU human chorionic gonadotropin (Sigma-Aldrich, St. Louis, Mo.) 48 h later (for superovulation) and then mated with male mice of the corresponding line. Donor dams were euthanized with carbon dioxide for embryo collection. Embryos were transferred into B6D2F1 female recipient mice that had been mated to vasectomized B6D2F1 male mice and observed to have a vaginal plug 24 h after mating. All of these litters were in Group 1. These mice were housed in a room that did not have a history of endemic mouse coronavirus infection.

(ii) Cross-fostering. SLOW-1, SLOW-2, FAST-1, FAST-2, and B6D2F1 female mice were bred with males from the corresponding line. The presence or absence of a vaginal plug was documented every day for 1 week. When a vaginal plug was identified (or after 7 days, if no plug was noted), the dams were moved to maternity cages and given a Nestlet (Ancare, Bellmore, N.Y.) for environmental enrichment. After parturition, litters were assigned randomly to Group 2 (ACS line pups cross-fostered to B6D2F1 dams), Group 3 (ACS line pups cross-fostered to different dam of same ACS line), or Group 4 (pups remained with birth dam; no fostering occurs). Groups 1 and 2 were housed in a production colony room with a documented history of sentinels negative for the viruses listed previously. Groups 3 and 4 were housed in a production colony room with a documented history of mouse coronavirus infection.

For cross-fostering, the donor dam's litter completely replaced the recipient dam's existing litter. Entire litters were moved, and litter sizes were matched to within 2 pups to decrease the likelihood of maternal rejection of the new litter. All pups were fostered within 24 h of birth to a recipient dam with an age-matched litter, and cages were not disturbed for at least 72 h after transfer to reduce potential rejection.

Mouse coronavirus testing. From each treatment group, we collected 20 µl blood from the medial saphenous vein of each representative offspring at approximately 35 to 40 days of age. Because of the limited numbers of mice in Group 1 of SLOW-2 and Group 4 of FAST-2, individuals were sampled for mouse coronavirus and subsequently underwent behavioral testing. However, these mice were allowed 2 weeks to recover from the stress of the blood collection. The mouse coronavirus assay was performed in-house by using the Immunocomb (Charles River, Wilmington, Mass.). After blood collection, all offspring that had been selected for behavioral testing were moved to an experimental holding room for acclimation.

Behavioral testing. Individual animals from each rearing treatment group underwent activity assessment at 53 to 66 days of age. At least 11 animals were available for testing in each treatment group for each line and replicate (Table 1). The same procedures used during selective breeding of the FAST and SLOW lines (9, 24) were used during this study. On test day 1, mice received an intraperitoneal injection of 2 g/kg of ethanol (20%, v/v) and were placed in one of eight 40 cm × 40 cm Accuscan activity monitors (Columbus, Ohio) beginning 2 min after injection. The circular Lehigh Valley activity monitors were replaced several years ago with the Accuscan equipment, because of the age of the Lehigh Valley equipment and the difficulty in obtaining replacement parts for repair. Reliable line differences for responses to ethanol stimulation have been seen using the Accuscan devices across the several years since their replacement (23, 25). Eight equidistant pairs of photocell beams and detectors mounted 2 cm above the test chamber floor detected locomotion. Beam interruption data were translated by computer to distance traveled in cm. Mice were tested for 4 min; 24 h later, all mice were injected intraperitoneally with normal saline and placed into the same Accuscan activity monitor in which they had been tested on day 1. Mice were again tested for 4 min beginning 2 min after injection. The Day 2 baseline score was subtracted from the Day 1 ethanol score to determine a within-

Table 1. Behavioral test groups and mouse coronavirus (MHV) test results

	FAST-1	FAST-2	SLOW-1	SLOW-2
Group 1: Embryo transfered	None available for testing	None available for testing	None available for testing	n = 14 mice 0/4 (0%) MHV-positive
Group 2: Fostered to B6D2F1	n = 21 mice 0/7 (0%) MHV-positive	n = 18 mice 0/6 (0%) MHV-positive	n = 25 mice 0/9 (0%) MHV-positive	n = 21 mice 0/5 (0%) MHV-positive
Group 3: Fostered within line	n = 20 mice 0/6 (0%) MHV-positive	n = 24 mice 5/9 (56%) MHV-positive	n = 25 mice 0/9 (0%) MHV-positive	n = 17 mice 2/5 (40%) MHV-positive
Group 4: No fostering	n = 24 mice 0/7 (0%) MHV-positive	n = 11 mice 5/5 (100%) MHV-positive	n = 24 mice 3/12 (25%) MHV-positive	n = 27 mice 6/16 (40%) MHV-positive

For SLOW-2 mice in Group 1 and FAST-2 mice in Group 4, those tested for MHV also underwent behavioral testing. For all remaining groups, offspring were tested for either MHV or behavior but not both.

group measure of ethanol stimulation for ethanol-treated mice, which we termed 'delta ACT'; this trait was used in the original selection of these lines and was thus the main dependent variable used here. Results initially were evaluated using analysis of variance (ANOVA) (Stastica version 6.1, Statsoft, Inc., Tulsa, Okla.) grouped on line (FAST or SLOW), replicate (1 or 2), and treatment group (1, 2, 3, or 4). However, each replicate set of lines (FAST-1 and SLOW-1 or FAST-2 and SLOW-2) also was examined in separate analyses to fully determine possible effects of these rearing conditions on behavior within each replicate. Newman–Keuls mean comparisons were used to evaluate differences between groups where applicable (Stastica version 6.1, Statsoft, Inc., Tulsa, Okla.).

Results

Reproductive manipulation. Of the four lines of mice, only the SLOW-2 mice were derived successfully using embryo transfer (Group 1). We suspected this difficulty was due to inappropriate temperature or composition of the collection media, rather than some association with inherent differences among the lines. Unfortunately, there were not sufficient mice available to repeat the embryo rederivation attempt with the other ACS lines. Multiple litters were produced from each line for Groups 2 through 4.

Mouse coronavirus testing. All mice in Groups 1 and 2 tested negative for mouse coronavirus. For Groups 3 and 4, approximately 30% of the tested mice had positive test results for mouse coronavirus (Table 1).

Behavioral testing. Delta ACT data from Groups 1 and 4 of SLOW-2 mice first were analyzed separately from all other data because SLOW-2 was the only line for which embryo transfer behavioral data were available. This analysis determined whether mice derived by embryo transfer (Group 1) differed from those reared under normal conditions (Group 4). There was no statistically significant effect of embryo transfer on the ethanol depressant phenotype of SLOW-2 mice ($P = 0.266$). There was a tendency for embryo-transferred mice to show a less extreme phenotype (less locomotor depression in response to ethanol), but this characteristic did not cause great concern because the expected line-typical phenotype was still robust (Fig. 1).

Next, data from Groups 2, 3, and 4 of all lines were analyzed by ANOVA grouped on line, replicate, and treatment group. A significant effect of line ($F[1,245] = 1228$, $P < 0.001$) demonstrated the marked difference between FAST and SLOW mice in locomotor response to ethanol. In addition, there was a trend toward a significant effect of treatment group ($P = 0.061$), a significant effect of replicate ($F[1,245] = 266$, $P < 0.001$), and a trend toward an interaction of line, replicate, and group ($P = 0.091$). Therefore, separate analyses were performed for each replicate set of lines.

For replicate 1 mice, there was a significant effect of line ($F[1,133] = 196$, $P < 0.001$) but no effect of group or interaction of line and group (Fig. 2A). Therefore, fostering had no statistically significant effect on the ethanol-stimulant phenotype of FAST-1 mice or the ethanol-depressant phenotype of SLOW-1 mice. For replicate 2 mice, there was again a significant effect of line ($F[1,112] = 1210$, $P < 0.001$). In addition, the effect of group approached significance ($F[2,112] = 2.96$, $P = 0.056$) as did the interaction of line and group ($F[2,112] = 2.86$, $P = 0.061$) (Fig. 2B). Although the interaction was not significant at the level of $P < 0.05$, follow-up analyses were performed within each line because there were a priori reasons to assess whether fostering had effects in specific lines. For the SLOW-2 line, there were no differences in locomotor responses to ethanol among groups. However, there were differences among groups for the FAST-2 line (simple main effect analysis, $P < 0.02$). Newman–Keuls mean comparisons indicated that non-fostered mice (Group 4) were significantly more stimulated by ethanol than were mice fostered on FAST-2 dams (Group 2; $P = 0.03$) or B6D2F1 dams (Group 3; $P = 0.002$). It should be noted that the smallest group size available was for FAST-2 Group 4 (n = 11). However, the standard error of the mean was comparable across the FAST-2 groups, indicating that the responses of these mice were not more variable than those of the others.

Discussion

As has been reported in the literature, fostering appeared to be a promising method to eradicate mouse coronavirus from an endemically infected colony (14, 17). Mice that had been cross-fostered to mouse coronavirus-free dams were found to be negative for mouse coronavirus antibodies. Unfortunately, the limited sample size of our study and other concerns preclude full endorsement of cross-fostering as an effective means of eradicating this virus. For example, the mouse coronavirus antibody status of the ACS dams (donor and recipient) was not obtained, so the timing and extent of exposure for the pups is undefined. However, the absence of mouse coronavirus antibodies in Groups 1 and 2 suggested to us that this method should be considered in the future, and our institution later used cross-fostering to successfully eradicate mouse coronavirus from a subset of the affected colony (14). Further, all ACS lines have remained coronavirus-free, as indicated by our established sentinel testing program.

Because Groups 1 and 2 were housed in an animal room free of endemic mouse coronavirus and Groups 3 and 4 were housed in an animal room with endemic mouse coronavirus, it is likely

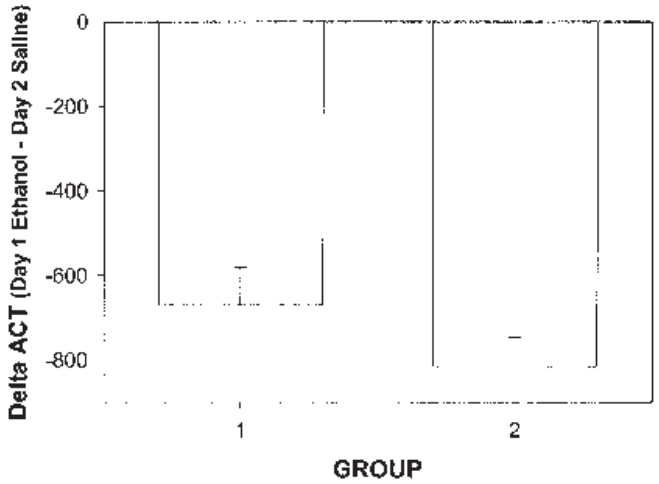

Figure 1. Locomotor activity responses of Group 1 (embryo-transferred) and Group 4 (non-fostered) SLOW-2 mice to 2 g/kg ethanol. Data are shown as mean ± SEM delta ACT score.

that cross-contamination occurred, leading to the positive results seen in Groups 3 and 4. Viral persistence was identified later in the replicate 2 mice (13) during the mouse coronavirus eradication efforts (14).

It was determined that fostering had a significant effect only on the ethanol response of the highly sensitive FAST-2 line. The ethanol-stimulant response of the two foster groups of this line, although reduced compared with that of the non-fostered group, was still quite robust, and we consider that this effect will not cause noteworthy difficulty if chosen as a means for eradication of mouse coronavirus. The fostering manipulation did not have a significant effect on the relevant behavioral trait in the other three lines.

We have not performed studies identical to those performed at the time of the rederivation of the FAST and SLOW lines. However, we have collected data pertinent to the stimulant response to ethanol in these lines. In general, we find that a robust difference continues to exist between the lines, with FAST mice displaying at least 6.6-fold greater stimulation to a 2-g/kg dose of ethanol compared with that of SLOW mice in recent studies (15). This degree of difference is similar to the approximately fivefold difference we saw in similar studies prior to rederivation (19). Therefore, the consequences of cross-fostering and presence or absence of mouse coronavirus appear inconsequential to the goals of our research, which are to identify the genetic and neurochemical mechanisms underlying differences in stimulant response to ethanol.

It is also promising that embryo transfer did not appear to have a significant effect on the phenotype of the SLOW-2 mice. However, because embryo transfer was only successful in this line, whether the same outcome would have occurred in the other three lines remains an important question. We decided we would need to repeat this study in the other three lines before proceeding with embryo transfer as a means to eradicate mouse coronavirus in these lines. In addition, it is crucial to determine the reasons for the failure of the embryo transfer procedure in the other three lines of mice. Technical error, mismatching of donors and recipients, and poor embryo production after superovulation were identified as potential causes of this failure. However, the possibility that some inherent difference in the three lines led to the poor success of this method cannot be ruled out.

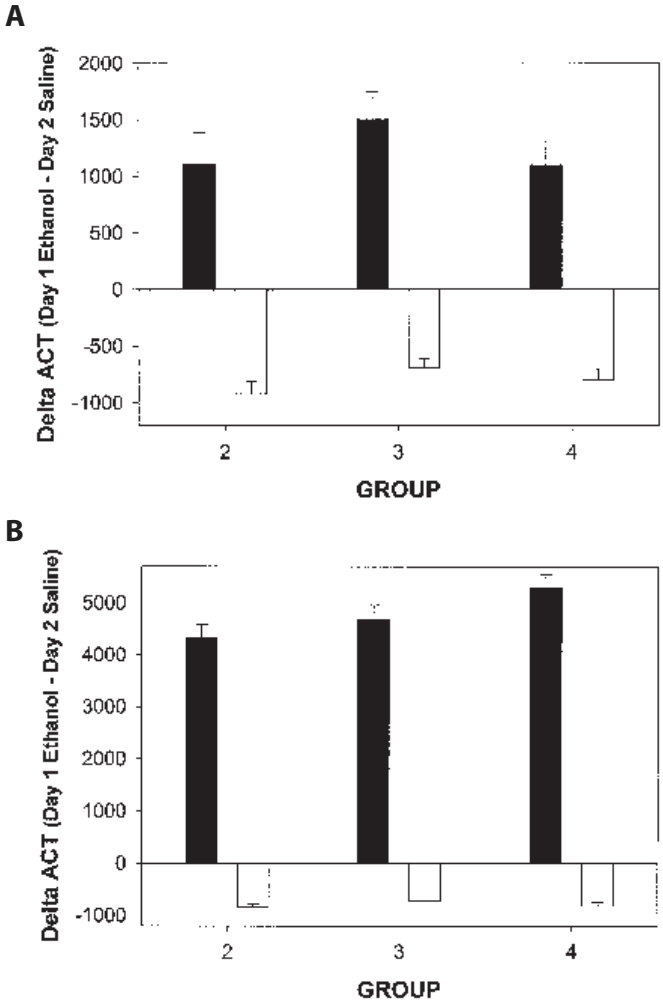

Figure 2. (A) Locomotor activity responses (delta ACT scores) of Group 2 (fostered to B6D2F1), Group 3 (fostered within line), and Group 4 (non-fostered) replicate 1 FAST (black bars) and SLOW (white bars) mice to 2 g/kg ethanol. Data are shown as mean ± standard error. (B) Locomotor activity responses (delta ACT scores) of Group 2 (fostered to B6D2F1), Group 3 (fostered within line), and Group 4 (non-fostered) replicate 2 FAST (black bars) and SLOW (white bars) mice to 2 g/kg ethanol. Data are shown as mean ± standard error.

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