Paternal Transmission of Complex Phenotypes in Inbred Mice

Mark D. Alter, Ahmed I. Gilani, Frances A. Champagne, James P. Curley, J. Blake Turner, and Rene Hen

Background: Inbred mice are genetically identical but nonetheless demonstrate substantial variability in complex behaviors such as activity levels in a novel environment. This variability has been associated with levels of parental care experienced early in development. Although maternal effects have been reported in biparental and uniparental strains, there have been no investigations of paternal effects in non-biparental strains in which offspring are reared exclusively by mothers.

Methods: In the uniparental inbred Balb/cJ mouse strain, we examined the relationship of paternal open-field activity to the activity of both male and female offspring in the open-field. Potential mediators of paternal transmission of behavior were examined, including maternal care, growth parameters, litter characteristics, and time the father was present with the pregnant mother prenatally.

Results: An association of paternal open-field activity with the open-field activity of female but not male offspring was found. Variation in maternal postnatal care was associated with female but not male offspring activity in the open-field but did not mediate paternal effects on offspring behavior. Paternal effects on offspring growth parameters were present, but these effects also did not mediate paternal effects on behavior.

Conclusions: Paternal transmission of complex traits in genetically identical mice reared only by mothers suggests a nongenetic mechanism of inheritance potentially mediated by epigenetic factors. The exclusion of multiple mediators of paternal effects on offspring suggests the possibility of germline paternal inheritance via sperm of complex phenotypes in inbred mice. Future studies are required to examine these interesting possibilities.

Key Words: Anxiety, Balb, epigenetic, inheritance, mouse, nongenetic, paternal transmission

Despite being genetically homogenous, inbred mice exhibit substantial and stable variability in complex phenotypes such as activity levels during a test of open-field exploration (1). Because they are genetically identical, inbred mice are well-suited for the study of nongenetic factors related to phenotypic variation. There is evidence from a number of experimental paradigms indicating that early life experience affects gene expression and behavior in rodents (1–14). In particular, variability in mother–infant interactions predicts variability in adult offspring on a number of behavioral measures, including the open-field test, and this variability is associated with variability in epigenetic modifications (3,4).

The contribution of maternal factors to offspring behavior is well-studied in both rats and mice (4,7,9–12,15–19). Although studies of paternal factors are few, there have been some reports from animal models of the nongenetic paternal transmission of phenotypes such as coat color and fertility (20,21). Human studies also suggest nongenetic paternal effects on the phenotypes of offspring. For instance, increased paternal age is a risk factor for schizophrenia, autism, and decreased IQ (22–30). In a pilot experiment we found that offspring from fathers separated by extreme differences in open-field behavior had behavioral differences in the open-field test resembling the behavioral differences of their fathers (Figure 1 in Supplement 1). Here we replicated these findings and tested the hypothesis that normal variations in paternal behavior in the open field before mating will predict normal variations in offspring open-field behavior in adulthood. We found this to be true only in female offspring and provide some preliminary evidence that pre- and postnatal effects might override paternal contributions in males. We also provide data to suggest that paternal contributions to offspring phenotype are pleiotropic and sexually dimorphic. The finding of nongenetic paternal contributions to offspring phenotypes has important implications for understanding complex inheritance patterns of psychiatric disorders and might provide a useful model for studying mechanisms underlying the origins of complex disease.

Methods and Materials

Animals

Balb c/J mice were used for all experiments (Jackson, Bar Harbor, Maine). Before mating and after weaning, mice were group-housed in sex-specific cages (5 mice/cage) and maintained on a 12-hour/12-hour light/dark cycle with food and water available ad libitum. All animal protocols were reviewed and approved as meeting appropriate ethical standards by Columbia University's and New York State Psychiatric Institute's Institutional Animal Care and Use Committee boards.

Mating

Ten-week-old male mice were housed with 2–3 females (10 weeks old) for 2 weeks and then were removed. To score maternal behavior specific to each litter, females were singly housed after 18 days after mating until delivery.

Paternal Behavior

Fathers were tested in a novel open-field test at 9 weeks of age (see following).

From the Division of Integrative Neuroscience (MDA, AIG, RH); Department of Psychiatry (MDA, AIG, JBT); Division of Child and Adolescent Psychiatry (MDA, JBT); and the Department of Psychology (FAC, JPC), Columbia University, New York, New York.

Address correspondence to Mark D. Alter, M.D., Ph.D., Division of Integrative Neuroscience, Columbia University, Room 767B Kolb Annex, 1051 Riverside Dr., New York, NY; E-mail: alterm@childpsych.columbia.edu.

Received Jul 1, 2008; revised May 19, 2009; accepted May 21, 2009.

0006-3223/09/$36.00
doi:10.1016/j.biopsych.2009.05.026

Maternal Behavior

Maternal behavior was scored 2 times/day during the light phase (9:00 AM and 1:00 PM) for a total of 50 measurements 1 min apart during each period (total of 100 measurements/day) for the first week of life. We find, as reported by others, maternal behaviors decrease during the first week of the postnatal period. Behavioral data were log transformed to obtain normal distributions.

Open-Field Behavior

Offspring were tested at 9 weeks of age. Activity in an open field is quantified in four Plexiglas open-field boxes 43 $cm^2 \times 43$ cm^2 with two sets of 16 pulse-modulated infrared photobeams (MED Associates, Georgia, Vermont). Data were analyzed on the basis of 2 zones: center (25% total area) and surround (75% total area). Behavioral data were log transformed to obtain normal distributions. In the case of offspring, mice were tested both under light (200 lux, first test) and on the following day in dark conditions (second test). Testing in the dark increases the spread of data by increasing the number of animals and amount of time that animals will spend in center regions. Light and dark measures are highly correlated, suggesting that both measures are testing similar constructs (1). In the present study, behavioral measures from the dark are used for analyses. Similar but weaker paternal effects were seen for measures of offspring behavior in the light.

Weight Measurements

Offspring were weaned at 4 weeks and weighed at this time. Adult body weight was measured at the time of death. Body weight was measured on a scale with sensitivity to .001 g. Brain weights were measured on a scale with sensitivity to .0001 g. Mice were killed by cervical dislocation; brains were removed and weighed. The brain was then hemisected, and the right and left hippocampus were removed and weighed individually. Total hippocampal weight was calculated by adding left and right measurements.

Statistical Analyses

Statistical analyses were performed with StatView software (College Station, Texas). To control for multiple testing, factor analysis and multiple regression analysis was used.

Factor Analysis

Open-field measures were highly correlated within individuals (Figure 1 in Supplement 1). Factor analysis was used to reduce five open-field behavioral measures (distance traveled in the center area [cen], percent of total distance traveled in the center area [% *p*], time in the center [time], entries into the center [ent], and total distance traveled [tp]) to a single latent variable. Open-field factor scores were used as a measure of open-field behavior for subsequent analyses.

Multiple Regression

Multiple regression was used to control for potential confounders and mediators of the associations we found. In each case we first created a model with a single dependent and independent variable. Next we added potential confounders to the model. Finally, we added potential mediators to the model containing potential confounders. Variables were median normalized and center on the mean before regression analysis.

Results

Paternal Contributions to Offspring Phenotype

There was a significant positive association of paternal behavior in the open-field test before mating with female but not male offspring behavior in the open field (Table 1). A number of potential confounders and mediators of this effect were entered into correlation matrices for female (Table 1) and male offspring (Table 1). The relationship of litter-specific variables to each other was also assessed (Table 1). In females but not males, there was a negative association of paternal open-field behavior with female hippocampus weight and a trend ($p = .1$) for a positive association of maternal arched-back nursing with open-field behavior. In males but not females, there were significant positive associations of paternal open-field behavior with body weight at weaning and brain weight in adulthood and a trend ($p < .1$) for an association with adult body weight. There was a trend ($p < .1$) for a positive association of maternal arched-back nursing with adult body weight. Litter size was positively associated with male but not female weight at weaning, adult body weight, and hippocampus weight. At the litter level there was a trend ($p < .1$) for an association of paternal open-field behavior with the percent females in a litter.

In sum the relationship of fathers' open-field behavior to offspring phenotype differed between male and female offspring with an increase in open-field behavior of fathers before mating predicting an increase in adult levels of open-field behavior in female but not male offspring and a decrease in the weight of the female offspring's hippocampus. Whereas in males, fathers' open-field behavior predicted differences in general growth parameters including increased weight at weaning, adult body weight, and total brain weight but no change in the weight of the hippocampus (Table 1). Additionally, male but not female offspring appeared sensitive to litter size with negative effects on open-field behavior and positive effects on weaning weight, adult weight, and hippocampus weight (Table 1).

Multiple regression analysis was performed in a stepwise fashion on the association of the open-field behavior of fathers and female offspring. First, maternal care including arched-back nursing and licking and grooming were entered into the model as possible confounders. The association remained significant (Table 2). Next, potential mediators were entered into the model. These included: maternal arched-back nursing, maternal licking and grooming, litter size, percent females in litters, weight at weaning, adult body weight, brain weight, and hippocampus weight. When all variables were entered into the model, the association of paternal open-field behavior with female offspring open-field behavior continued to be significant (Table 2). A similar analysis was performed on male offspring that indicated the association of litter size with male offspring behavior remained highly significant in the complete model (Table 2).

Germline Versus Prenatal Effects of Fathers on Offspring Phenotype

A possible explanation for paternal effects on offspring phenotypes was that fathers who behaved differently in the open field had different effects on the prenatal environment (Table 3). In our experimental design, fathers were housed with pregnant females for between 7 and 14 days after conception. We reasoned that an effect of fathers on the prenatal environment would be greater when the father was present for a longer period. To examine this we calculated the time that a father spent