Epigenetic sources of behavioral differences in mice

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Inbred mouse strains are classically used to search for the genes associated with behavioral traits, including emotionality. To distinguish genetic and environmental contributions to the expression of adult behavior in mice, we investigated the effects of prenatal (embryo transfer) and postnatal (cross-fostering) environments in two strains of inbred mice with profound and reliable differences in behavior[1]. Here we report that strain-related behavioral differences may result from environmental factors during development rather than genetic differences between the offspring.

C57BL/6J (B6) mice were cross-fostered at prenatal and postnatal time points to either BALB/cJ (BALB) or B6 dams (see **Supplementary Methods** online for details). Prenatal cross-fostering was performed by removing single cell pronuclei from B6 females 12–16 hours after mating for implantation in pseudopregnant BALB or B6 foster dams. Postnatal cross-fostering was performed within 12 hours of delivery by transferring newborn pups from these litters to either BALB or B6 parturient females. Litters consisted of 8 pups per dam, with an equal number of males and females. All pups were cross-fostered, and all litters were composed of pups from all developmental histories. Thus we created four developmental conditions for the genetic B6 offspring (with a minimum of 8 subjects per group): prenatal BALB/postnatal BALB, prenatal BALB/postnatal B6, prenatal B6/postnatal BALB and prenatal B6/postnatal B6. There were no differences in mean litter size (B6, 8.00 ± 0.62 pups; BALB, 8.50 ± 0.65 pups) or mean litter weights at birth (B6, 1.42 ± 0.02 g; BALB, 1.49 ± 0.05 g). Ten control B6 and BALB males obtained directly from the breeders were used to define strain differences in exploratory behavior, anxiety-related behavior, water maze performance and sensorimotor gating[2,3]. These two control groups were included to define the expected phenotypic differences, as varying B6 and BALB mouse substrain differences have previously been reported.

When tested at 3 months of age, control B6 and BALB males showed significant differences in exploration of an open field, relative time on the open arms of a plus maze, latency to find a hidden platform in the Morris water maze (MWM) and acoustic startle pre-pulse inhibition (PPI) (**Fig. 1a**, **c**, **e** and **g**). In experimental animals, either prenatal or postnatal cross-fostering to B6 dams did not have any apparent effect on these behaviors. However, B6 mice developing in a BALB uterus and reared by a BALB

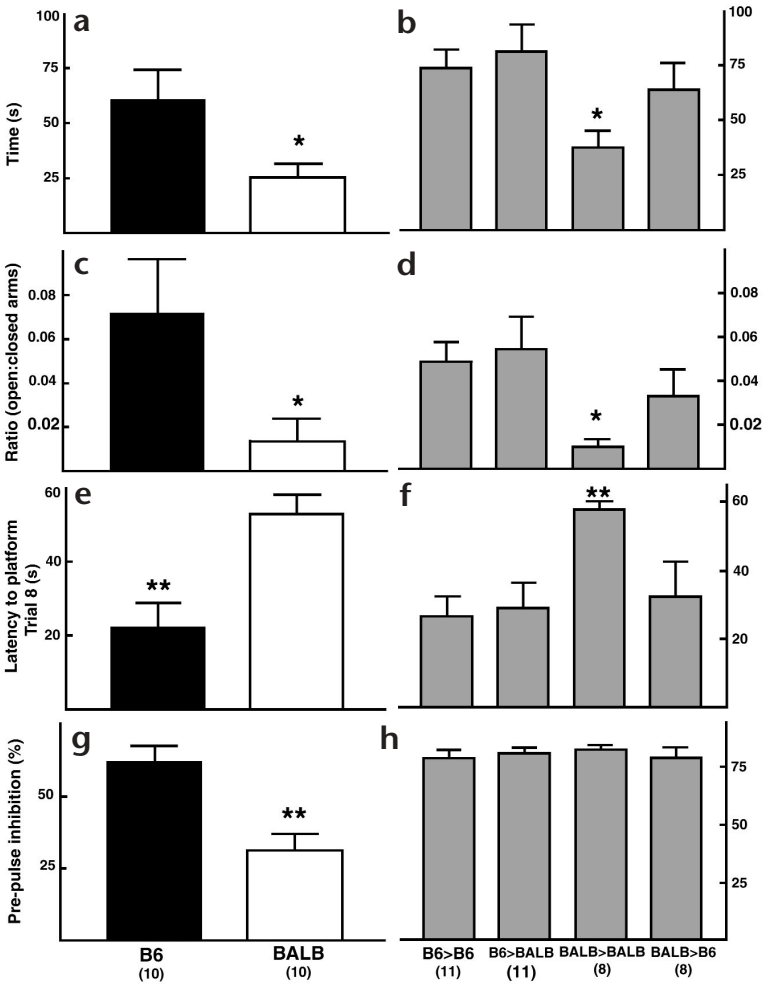

Fig. 1. Open-field, elevated-plus maze, water-maze learning and pre-pulse inhibition behavior. Inbred male B6 and BALB mice (non-fostered) differed on (**a**) mean time spent in the inner area of a novel open field (t_{18} = 2.26, P < 0.05), (**c**) mean ratio of time spent on the open arms of the plus-maze relative to the closed arms (t_{18} = 2.25, P < 0.05), (**e**) mean latency to find platform on trial 8 (note no group differences on trial 1) of water maze (t_{18} = 3.61, P < 0.01) and (**g**) mean percent inhibition of acoustic startle reflex with pre-pulse of 73 dB (t_{18} = 3.64, P < 0.01). B6 mice cross-fostered prenatally and/or postnatally to B6 dams (B6>B6, BALB>B6, B6>BALB) resemble control B6 mice on (**b**) open field, (**d**) plus maze, (**f**) Morris water maze, and (**g**) PPI behavior. B6 mice cross-fostered prenatally and postnatally to BALB dams (BALB>BALB) differ on (**b**) open field, (**d**) plus maze and (**f**) water maze behavior (*P < 0.05 in a *post-hoc* Dunnett's test between BALB>BALB and B6>B6 in the presence of a significant group effect by ANOVA). In contrast to these measures of open field, plus maze and water maze behavior (**h**), cross-fostering does not appear to alter PPI response, a measure of sensorimotor gating. All experimental protocols were approved by the Emory University IACUC. Bars are mean ± s.e.m.

mother showed behaviors on the open field, plus-maze and MWM that were identical to those of BALB mice and significantly different from other B6 mice. As all experimental animals were genetically identical B6 mice, these behavioral differences must result from non-genetic factors. This apparently epigenetic effect did not result entirely from prenatal factors, as mice developing in a BALB uterus but reared by a B6 dam did not show the BALB behavioral phenotype (**Fig. 1b**, **d** and **f**). In contrast to these behavioral measures, PPI performance was not different across experimental conditions (**Fig. 1h**). Interestingly, the control strain differences we report for PPI differ from those previously reported[4], probably because of differences in the substrains of mice used.

For decades, researchers have attempted to address the contribution of maternal factors to the development of behavior in offspring. Earlier studies using ovarian transplantation with similar substrains of mice were limited in experimental design by histocompatability differences between the strains, but nevertheless concluded that the maternal environment accounts for only a small proportion of the total variance in open-field behavior[5]. Subsequent studies using neonatal embryo transfers or postnatal cross-fostering have used different inbred strains of mice and focused on different phenotypic measurements such as growth patterns and reflex responses[6,7]. These studies suggest that the maternal environment can contribute to the phenotype of the offspring through various mechanisms[8]. Our current observations suggest that the prenatal environment interacts with the postnatal environment to shape the development of select adult behaviors. One potential postnatal mechanism that may contribute to the final phenotype is a difference in the maternal care received[9,10], as B6 and BALB dams provided different levels of maternal care. During the first five postnatal days, B6 dams licked pups more frequently ($16.20 \pm 0.66\%$ of observations taken every 2 minutes during 4 hours of observations) than BALB dams ($6.63 \pm 0.87\%$; $t_9 = 8.71$, $P < 0.0001$). Maternal licking in rodents has a regulatory role in the development of the endocrine–stress axis as well as exploratory behavior and maze learning[11,12].

In summary, these results indicate that some, but not all, of the stable behavioral differences between inbred strains may be due to epigenetic factors, such as the cascading role of the prenatal environment in concert with differences in postnatal rearing environments. Note, however, that our results are limited to genetically B6 mice; BALB embryos were not cross-fostered in this study. Previous studies in rats have found long-term, intergenerational consequences of individual differences in maternal care[13], providing a non-genomic mechanism for the transmission of behavioral traits. These new results, in mice, suggest that the prenatal environment may prime the developing pup to respond to postnatal care, such that a strain-specific phenotype develops independent of genotype. The precise nature of these prenatal influences remains to be determined.

Note: Supplementary information is available on the Nature Neuroscience website.

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Competing interests statement

The authors declare that they have no competing financial interests.

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Sound-induced differential motion within the hearing organ

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Hearing depends on the transformation of sound-induced basilar membrane vibration into deflection of stereocilia[1] on the sensory hair cells, but the nature of these mechanical transformations is unclear. Using new techniques to visualize and measure sound-induced vibration deep inside the moving organ of Corti, we found that two functionally crucial structures, the basilar membrane and the reticular lamina, have different centers of rotation, leading to shearing motion and rapid deformation for the mechanoreceptive outer hair cells. Structural relations within the organ of Corti are much more dynamic than previously thought, which clarifies how outer hair cell molecular motors can have such a powerful effect.

The high sensitivity and frequency selectivity of the hearing organ (**Fig. 1a**) is dependent on a specialized motor protein, prestin[2], which is localized to the outer hair cells (OHC). Force generated by prestin profoundly affects organ vibration. Consequently, mice lacking this protein have greatly reduced hearing ability[3]. Classically, the organ of Corti has been assumed to vibrate as a stiff unit, without structural changes, around the point where the basilar membrane attaches to the bony core of the cochlea[4] (asterisk in **Fig. 1b**). Indirect experimental data[5,6] support this idea, but a fundamental question remains: how can OHC molecular motors have such a large effect if the structure remains unaltered? One potential solution, also supported by indirect data[7–10], is that the OHCs deform such that the basilar membrane and the