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Transgenerational effects of impaired maternal care on behaviour of offspring and grandoffspring

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Cues received from mothers may have important effects on early development in mammals. We examined the behavioural development of genetically wild-type mice, *Mus musculus*, offspring born to wild-type or mutant Peg3$^{+/-}$ (paternally expressed gene 3) mothers who are impaired in various aspects of maternal care during both the pre- and the postnatal periods. We demonstrate that impaired maternal care leads to offspring exhibiting increased neophobia and decreased exploratory behaviour. However, these effects were limited to female offspring only, with male offspring being indistinguishable from wild types in both the open-field and the novel object tests. Due to the breeding design of this study we were able to show that this was due not to the inheritance of a genetic mutation but to an epigenetic inheritance. Consistent with this, we also observed a nongenomic inheritance of impairments in maternal care. Wild-type daughters born to mutant mothers were impaired in their ability to retrieve pups to a nest in a retrieval test. Furthermore, the reduced exploration and neophobic phenotypes were transmitted to a third generation, with the granddaughters of mutant females exhibiting increased neophobia even though they had genetically wild-type mothers. We therefore demonstrate a nongenomic transmission of behavioural traits across successive generations operating via the matriline.

Keywords: developmental plasticity; epigenetics; exploration; genomic imprinting; maternal care; maternal effect; *Mus musculus*; novelty; parent-of-origin effects; transgenerational inheritance

In mammals, females are the primary caregivers and provide developing organisms with cues about how best to develop. Evidence from human and other animal studies shows that aversive events occurring early in life can have long-term effects on offspring phenotype. In humans, neglect and abuse in infancy increase risk of depression and anxiety and lead to metabolic disorders (Susser & Lin 1992; Bateson 2001; Bateson et al. 2004; Gluckman et al. 2005). Infant rhesus monkeys, *Macaca mulatta*, reared in the absence of their mothers, are behaviourally inhibited, show increased responsivity to stress and display impairments in social and reproductive behaviour as adults (Suomi et al. 1971; Ruppenthal et al. 1976). In rodents, daily separations of litters from their mothers during the postnatal period lead to long-lasting changes in cognitive, anxiety, reproductive and social behaviours in offspring, associated with gene expression changes in numerous brain regions (Fleming 1975; Lovic et al. 2001). Even relatively brief separations where pups are handled daily during the first week postpartum for 3–15 min can lead to long-lasting changes in stress reactivity and fearfulness even up to 26 months of age (Levine 1957; Meaney et al. 1988). Moreover, exposure to disruptive events during gestation, such as various stressful episodes or food restriction, induces the development of similar impairments in offspring behaviour (Salas et al. 1984; Champagne & Meaney 2006). Fewer studies have focused on more subtle variations in the mother–infant relationship. Nevertheless, in rodents and primates individual differences in the levels of postnatal contact or tactile stimulation of offspring by mothers may lead to these offspring developing alternative social, cognitive, parental and stress phenotypes (Fairbanks & McGuire

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1988; Champagne et al. 2001). Finally, studies in mice using reciprocal hybrids and embryonic transfer have revealed that differences in the prenatal environment may also shape the offspring's adult phenotype (Calatayud & Belzung 2001; Francis et al. 2003; Calatayud et al. 2004; Caldji et al. 2004; Priebe et al. 2005). In these studies, changes in stress reactivity, exploratory behaviour, response to novelty and maternal behaviour of offspring in response to the maternal environment are observed (Champagne & Curley 2005).

The main focus of the above approaches has been to demonstrate maternal effects on offspring phenotype that occur over one generation. However, life history theory of evolutionary biology strongly suggests transgenerational transmission of phenotypes including behaviour (Jablonka & Lamb 2005). Since grandoffspring may inherit the physical or social environment of their parents and grandparents, it could be beneficial for them to inherit the phenotype and hence epigenetic status of their parents and grandparents also. Evidence supporting this hypothesis comes from the nonmammalian evolutionary biology literature, particularly in *Drosophila* where various environmentally induced morphological variants are able to transmit their phenotype nongenomically via the mother (Rogilds et al. 2005). Such transgenerational maternal effects on offspring morphology and reproductive success have also been reported in vertebrates such as fish (Bashey 2006) and birds (Naguib et al. 2006). In mammals, the possibility for transgenerational maternal inheritance of behavioural phenotypes has not been studied as extensively though examples of these effects have been observed in rats. Meaney and colleagues (Champagne et al. 2003a) have demonstrated that individual differences in the licking and grooming of offspring by rat dams lead to altered behaviour in both male and female offspring and grandoffspring. Through cross-fostering, it has also been demonstrated that the effect of handling pups on their response to novelty as adults can be inherited in a nongenomic fashion across generations (Denenberg & Rosenberg 1967).

In our previous studies we showed that mutant Peg3$^{+/-}$ females are impaired in various components of their maternal care including the supply of nutrients to the developing embryo prenatally and the nursing, retrieval and licking/grooming of pups and nest building postnatally and also that they are neophobic (Li et al. 1999; Curley et al. 2004; Champagne et al. 2005). The *Peg3* gene is imprinted and is expressed in an individual only when it is inherited from the father because the maternal allele is always silenced (Li et al. 1999). It encodes a zinc finger protein that regulates cell survival in the placenta, embryo, and developing and adult brain and therefore has the capacity to shape and remodel offspring development and behaviour (Kuroiwa et al. 1996; Relaix et al. 1998; Li et al. 1999; Deng & Wu 2000; Johnson et al. 2002; Swaney et al. 2007). Mechanistically, the deficits observed in Peg3$^{+/-}$ mutant females appear to be related to impairments in the oxytocinergic system, with mutants possessing fewer oxytocin neurons in the paraventricular and supraoptic nuclei of the hypothalamus and fewer oxytocin receptors in the medial preoptic area (Li et al. 1999; Champagne et al. 2005). Given that mutant Peg3$^{+/-}$ females exhibit these phenotypic differences, we investigated whether their offspring also exhibited changes in exploratory behaviour and maternal behaviour and whether any changes could be transmitted to a third generation. We chose to use two tests (novel object and open field) that investigated both aspects of the approach (exploration) versus withdraw (neophobia) conflict that occurs when an animal is faced with novelty (van Gaalen & Steckler 2000; Tang & Sanford 2005) and the retrieval test to measure maternal care (Champagne et al. 2007). Significantly, as the *Peg3* mutation is expressed only when inherited through the patriline, we were able to investigate the behaviour of genetically wild-type (WT) offspring and grandoffspring that differed only with respect to the quality of the maternal environment during the pre- and postnatal periods (Fig. 1).

METHODS

Subjects

All procedures were undertaken with the relevant ethical approvals, including a review by the University of Cambridge Animal Ethics Committee, a Home Office project licence to E.B.K., as well as Home Office personal licences to E.B.K. and J.P.C. All subjects were laboratory mice, *Mus musculus*. Animals were housed at the Sub-Department of Animal Behaviour (Madingley, Cambridge, U.K.) on a reverse 12:12 h light:dark cycle, with the dark phase commencing at 0800 hours and the light phase at

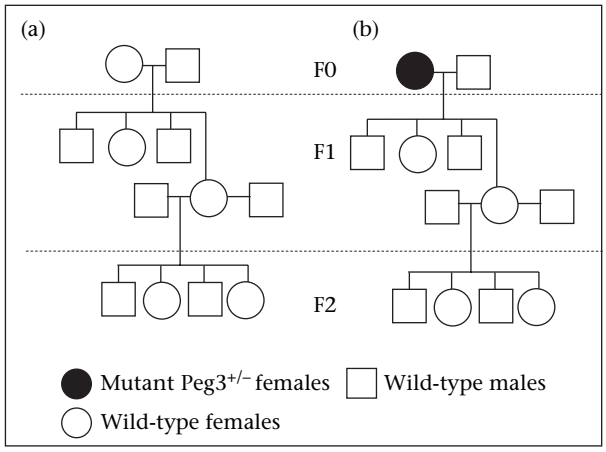

Figure 1. Breeding design. In the parental generation F0, 129Sv wild-type males are mated with either (a) 129Sv wild-type dams or (b) mutant 129SvPeg3$^{+/-}$ dams. Peg3 is an imprinted gene expressed according to parent of origin. Because the heterozygous Peg3$^{+/-}$ female is mated with a wild-type male, none of the F1 or F2 generations expresses this mutation. Hence epigenetic transgenerational effects of reduced maternal care can be determined through these generations. Virgin F1 offspring were behaviourally assessed in the novel object and open-field tests. Twelve F1 females in both groups were not used in these tests but were mated twice with 129Sv wild-type males to assess their maternal behaviour in a retrieval test with their first and second litters. The offspring of the first of these matings were weaned to produce the F2 generation and were tested as virgin animals on the novel object and open-field tests.