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REVIEW ARTICLE

Epigenetic Programming of Phenotypic Variations in Reproductive Strategies in the Rat Through Maternal Care

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Studies across multiple organisms reveal considerable phenotypic variation in reproductive tactics. In some species, this variation is associated with maternal effects in which variation in maternal investment results in stable individual differences in reproductive function. Recent studies with the rat suggest that maternal effects can alter the function of neuroendocrine systems associated with female sexual behaviour as well as maternal behaviour. These maternal effects appear to be mediated by epigenetic modifications at the promoter for oestrogen receptor alpha (ERα) and subsequent effects on gene expression. The tissue-specific nature of such effects may underlie the co-ordinated variation in multiple forms of reproductive function, resulting in distinct reproductive strategies.

Key words: estrogen receptor alpha, medial preoptic area, oxytocin, gonadotropin releasing, hormone, female.

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In the hunt for more reliable genotype–phenotype relations, few examples would seem as potentially rewarding as that describing the Y chromosome effect on gonadal differentiation in mammals. Thus, genetic sex (XX or XY) determines gonadal phenotype, from which derives the process of sexual differentiation into the male or female phenotype, or gender (1–4). However, a preoccupation with sexual differentiation neglects the intriguing issue of individual differences in the expression of traits directly associated with reproduction across members of the same sex (5, 6). We might assume that more complex functions, such as behaviour, admit more readily to variation between individuals of the same species (although variations in form across members of the same species have been underestimated; e.g. 7). Indeed, the pathways that lead from gonadal differentiation to differentiation of the HPG axis and behaviour are subject to considerable regulation, and thus variation in sexual differentiation within members of the same gender should not be surprising.

It is commonly thought that the capacity for phenotypic plasticity evolved to permit diversity in genotype–phenotype relations in response to variations in the level of environmental demands (7, 8). Such phenotypic diversity is likely to reflect complex gene–environment interactions involving protein–DNA interactions at sites (e.g. promoters, enhancers and suppressors) that regulate gene expression. It is interesting that, across species, increasing complexity is associated more with the size of the non-coding region of the genome than with the number of genes. We presume that this difference reflects the increased complexity of the regulatory regions of the DNA that, in turn, confers enhanced capacity for tissue-specific regulation of gene expression in multi-organ animals. In addition, the increased size of the regulatory region of the genome should also correspond to an increased capacity for environmental regulation of gene expression – a process whereby an increasing range of phenotypes might emerge from a common genotype: an increased capacity for phenotypic plasticity.

Parental effects on life history strategies

Variations in life history strategies among individuals within a species are often defined by adaptations to environmental conditions prevailing during early development, and reflect the remarkable capacity for phenotypic plasticity. Research in evolutionary biology reveals so-called 'parental effects' as a major source of phenotypic plasticity (8–13). Within evolutionary biology, parental effects are defined as sustained influences on any component of the phenotype of the offspring that is derived from either the mother or the father, apart from nuclear genes (10). Such parental effects have been described across a variety of species ranging literally from plants to mammals. Together, these studies suggest that the quality

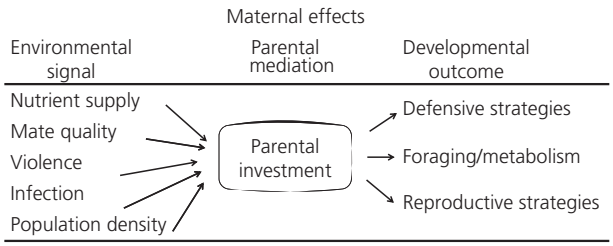

Fig. 1. A summary of the evolutionary biology literature on 'maternal effects' reflecting the common theme that environmental signals alter multiple phenotypic outcomes through effects on parent–offspring interactions ('parental investment'), the form of which will vary depending upon the species. The principal idea is that of parental mediation and of co-ordinated effects on multiple phenotypic outcomes. For example, in the ground-nesting bee, large larvae develop into males expressing a fighter phenotype that is flightless, has large mandibles, and mates within the nest (15). Smaller larvae develop into smaller males with functional wings that mate outside the nest. Maternal provisioning (a form of parental investment) determines male larval size and thus adult reproductive tactic as well as foraging strategies. The size and quality of the maternal provision (or propagule) among insects is commonly associated with the quality of the prevailing environment. This suggests that environmental conditions such as nutrient availability can influence the development of reproductive tactics through effects on parental investment.

of the prevailing environment, defined primarily as the abundance of nutrients and the risk for mortality, directly influences parent–offspring interactions that, in turn, influence the life history strategies of the offspring (Fig. 1). As Hinde (14) suggests, such effects are probably attributable to the fact that natural selection shaped offspring to respond to subtle variations in parental behaviours as a forecast of the environmental conditions in adulthood. This position implies adaptive value. Thus, Rossiter (10) suggests that natural selection favours parental effects that 'prepare' offspring for the expected environment *even if that environmental condition is one of uncertainty* (p. 130).

Maternal care in the rat

Maternal care in the rat involves the maintenance of the nest site and frequent nursing bouts. Milk delivery is the hallmark of the nursing bout; however, maternal licking/grooming (LG) of the pups is an important component of the nursing bout. Pup LG may be considered as a rudimentary form of parental 'nurturance', as this maternal behaviour serves to enhance somatic growth and brain development through immediate effects on multiple endocrine systems (16, 17).

There is considerable variation among lactating Long-Evans rats in the frequency of pup LG over the first week of life (18–22). Such variations permit the characterisation of high-and low-LG mothers (21). Lactating females for which the LG score is 1 standard deviation (SD) greater than the mean of the breeding cohort are deemed to be high-LG mothers. Conversely, those for which the LG scores are 1 SD below the mean are considered to be low-LG mothers. High-LG mothers commonly engage in pup LG at about twice the frequency as low-LG mothers, and this difference persists only for the first week of life (20, 21). The individual differences in the frequency of pup LG between the high- and low-LG mothers are stable across multiple litters (21) and represent a stable individual difference in a specific form of reproductive behaviour (Fig. 2).

The inter-generational transmission of individual differences in maternal behaviour

Individual differences in the frequency of pup LG can be transmitted across generations in the rat through a non-genomic, behavioural mode of 'inheritance'. Thus, the lactating female offspring of high-LG mothers show significantly increased levels of pup LG compared with those reared by low-LG dams (23, 24). Cross-fostering reverses the pattern of inheritance. The adult, lactating females born to low-LG mothers, but reared by high-LG dams, show increased pup LG compared with those reared by low-LG mothers (and *vice versa*). Similarly, the female offspring of high-LG mothers show increased maternal responsivity (i.e. a shorter latency for the onset of maternal behaviour) in the pup sensitisation paradigm (24).

These findings suggest that maternal–infant interactions may programme specific forms of reproductive behaviour. As lactating mothers, female rats artificially reared in isolation with no maternal care following the first day of life show significantly reduced responsiveness to pups and reduced pup LG (26, 27). The effects of artificial rearing on maternal behaviour are greatly reduced by providing the female pups with stroking, which mimics the tactile stimulation associated with maternal LG, as well as with social contact with peers. These findings suggest a direct relation between the quality of maternal care in early life and that expressed in adulthood (22, 28). The inter-generational transmission of individual differences in maternal behaviour is also apparent in primates (29–34).

Individual differences in maternal behaviour in the rat are associated with the quality of the environment. Thus, chronic stress dur-

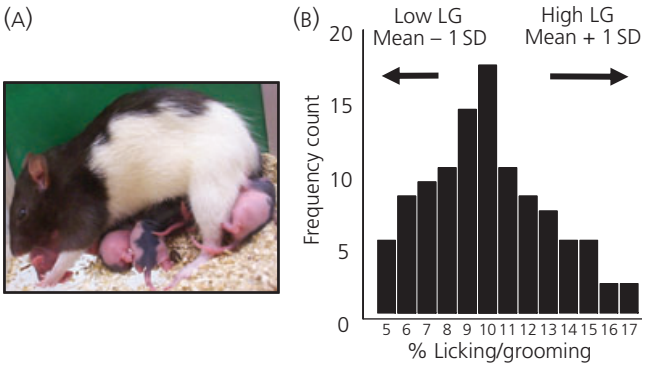

Fig. 2. (A) Photo depicting a mother engaged in pup licking/grooming (LG) during a nursing bout. (B) Frequency distribution of cumulative pup LG during the first 6 days postpartum (adapted from Ref. 23). Lactating females for which the mean frequency of pup LG from standardised behavioural observations were 1 standard deviation (SD) or more above the mean for the breeding cohort (60 to 68 litters) were designated high-LG mothers; those for which the pup LG scores were 1 SD or more below were designated low-LG mothers.

ing gestation decreases the frequency of pup LG (35, 36). Moreover, when exposed to stress during the last trimester of gestation, females previously characterised as high LG subsequently show levels of pup high LG indistinguishable from that of low-LG mothers (36). The lactating female offspring of these mothers also show decreased pup LG (36).

Neuroendocrine mechanisms

The elaborate pattern of maternal care that is evident shortly after parturition in the rat is orchestrated through endocrine events occurring during late gestation. Progesterone levels fall just days before parturition, followed by a surge in oestrogen levels (25). Both events are obligatory for the onset of maternal behaviour, and of particular importance are the effects of oestrogen at the level of the medial preoptic area (MPOA), which is critical for the expression of maternal behaviours in the rat (37, 38).

The influence of ovarian hormones on the onset of maternal behaviour in the rat is mediated, in part, by effects on central oxytocinergic systems (39). Oestrogen increases oxytocin synthesis in the parvocellular neurones of the hypothalamus (PVNh) that project to the MPOA as well as other brain regions that regulate maternal behaviour (40). Oestrogen also increases oxytocin receptor gene expression and receptor binding in the MPOA (e.g. 24, 41–43) and this effect appears to be mediated through the oestrogen receptor alpha (ERα; 44). Intracereboventricular (ICV) administration of oxytocin rapidly stimulates maternal behaviour in virgin rats (45, 46) and the MPOA appears to be a critical site. This effect of oxytocin is oestrogen dependent. The influence of oxytocin is abolished by ovariectomy and re-instated with oestrogen treatment. Moreover, treatment with oxytocin antisera or receptor antagonists blocks the effects of ovarian steroid treatments on maternal behaviour (46, 47). Among lactating females, there are significantly higher levels of oxytocin receptors in the MPOA (39). This lactation-induced increase in oxytocin receptor levels is substantially greater in the high-LG mothers (24, 48) and oxytocin receptor binding in the MPOA is highly correlated with the frequency of pup LG (49). Importantly, central infusion of an oxytocin receptor antagonist on day 3 of lactation completely eliminates the differences in pup LG between high- and low-LG mothers (24). Activation of the oxytocin receptor on oxytocin neurones, such as those of the MPOA, commonly results in an increase in oxytocin expression (a positive, feed-forward effect; 50, 51). There is increased oxytocin mRNA in the PVNh and supraoptic nucleus (SON) of the high-LG mothers (52), and both regions project to the MPOA (53). Predictably, there is also an increased number of oxytocin-positive cells in the MPOA of high- compared with low-LG mothers (52).

The ascending mesolimbic dopamine appears to be a relevant target for the effects of oxytocin on maternal behaviour (54). Oxytocin neurones in the MPOA project directly to the ventral tegmental area (VTA) (37, 52), the origin of the mesocorticolimbic dopamine system. Dopamine levels in the nucleus accumbens (nAcc) are increased during a nursing bout (55), and specifically during a period of pup LG (56). Dopamine levels in the nAcc are directly related to the frequency of pup LG (56). Dopamine levels in the nAcc during pup LG are greater in high- compared with low-LG mothers, and infusion of a dopamine re-uptake blocker, which eliminates the differences in nAcc dopamine levels, completely abolishes the differences in pup LG between high- and low-LG dams (56). These findings are consistent with earlier reports that lesions of the nAcc significantly reduce pup LG (36). Infusion of oxytocin into the ventral tegmental area (VTA) enhances dopamine release in the nAcc (D. Shahrokh, T-Y Zhang, J. Diorio, A. Gratton and M.J. Meaney, unpublished data). Finally, the differences in nAcc dopamine levels between high- and low-LG mothers during a period of pup LG are eliminated with the infusion of an oxytocin receptor antagonist into the VTA (D. Shahrokh, T-Y Zhang, J. Diorio, A. Gratton and M.J. Meaney, unpublished data). Taken together, these findings suggest that oestrogen effects, mediated through the ERα, enhance the sensitivity of the MPOA neurones to oxytocin by increasing oxytocin receptor binding, resulting in an increase in oxytocin expression and greater oxytocinergic signalling at the level of the VTA. The lynchpin for this cascade is the ERα expression in the MPOA.

Oestrogen receptor expression

Differences in oestrogen sensitivity mediate the differential effects of lactation on the induction of oxytocin receptors in high- and low-LG females. Among ovariectomised females provided with oestrogen replacement, there is a significantly greater oestrogen effect on oxytocin receptor levels in the MPOA in the female offspring of high-LG animals compared with those of low-LG animals (24). Similarly, ERα activation stimulates Fos expression, and there is greater oestrogen-stimulated Fos expression in the MPOA of the female offspring of high-LG mothers compared with low-LG mothers (43). The fact that such differences occurred even in the non-lactating, ovariectomised state suggests the existence of stable differences in oestrogen sensitivity. Indeed, among either lactating high-LG mothers or the virgin female offspring of high-LG dams, the expression of ERα, but not ERβ, is significantly increased in the MPOA (43). The effect is apparent at the level of both mRNA and protein.

The current working hypothesis suggests that the increased pup LG of high-LG mothers is associated with stable increases in ERα expression in the MPOA. During gestation, the increase in circulating oestrogen appears together with the increased ERα expression which enhances oxytocin receptor binding to a greater extent in the high-LG mothers, leading to increased oxytocin activation of the ascending mesolimbic dopamine system and increased dopamine release in the nucleus accumbens. According to this model the critical feature for the transmission of the individual differences in maternal behaviour from the mother to her female offspring is the differences in ERα expression in the MPOA.

Maternal effects on HPG function and mating behaviour

To examine the regional specificity, we examined ERα expression in multiple brain regions. To our surprise we found that ERα mRNA expression is significantly increased in the anterioventral paraven-

tricular nucleus of the hypothalamus (AVPVn; 57) as well as in the ventromedial nucleus of the hypothalamus (VMHn) in the *offspring of low-LG mothers*. Oestrogen acts through oestrogen receptors in the AVPVn to regulate gonadotrophin-releasing hormone (GnRH) with downstream effects on pulsitile luteinising hormone (LH) and ovarian hormone production (e.g. 58–61). The differences in ERα expression in the AVPVn appear to be functionally relevant. In intact cycling adult females, circulating levels of LH at pro-oestrous are significantly higher in offspring of low-LG mothers than in those of high-LG mothers (57). Similarly, there are increased pro-oestrous levels of progesterone in the low-LG offspring. The female offspring of low-LG mothers show increased sensitivity to the positive feedback effects of oestrogen on neural systems that regulate LH release. Thus, among ovariectomised animals, oestrogen administration produces a significantly greater increase in GnRH expression and plasma LH levels in the offspring of low-LG dams than in those of high-LG dams (57).

Because oestrogen and progesterone regulate mating over the oestrous cycle in the rat, these findings suggest possible effects of maternal care over the first week of life on sexual behaviour in the female offspring. Females were tested at pro-oestrous with males in the confinements of a smaller, traditional testing arena (57). Under these circumstances the female offspring of low-LG mothers exhibited an increased lordosis response to male mounts as well as increased rates of proceptive behaviours (63) that serve to attract the male and enhance male copulation. In contrast, the female offspring of high-LG dams exhibited increased levels of agonistic behaviour towards the males.

When a receptive female is tested in a larger area that affords the opportunity to retreat from the male, an approach-withdrawal pattern prevails and reveals the females' ability to pace the mating with the male (62). Female rats pace the rate of male intromissions and thus ejaculation by withdrawal from the male following each intromission. The latency to return to the male is longer after ejaculation than after an intromission, which, in turn, is longer than after a mount with an intromission (62–67). As testing proceeds over the courses of multiple ejaculatory sequences, the inter-intromission interval increases (62). Testing in the pacing chamber revealed considerable differences in sexual behaviour as a function of maternal care (57, 67). As in the previous test, the critical measure of receptivity (lordosis rating) suggested *decreased sexual receptivity* in the adult female offspring of high-LG mothers by comparison to those reared by either low- or mid-LG dams. Over the entire session the average inter-intromission interval was substantially longer in the female offspring of high-LG mothers. Importantly, there were also significant group differences in the rate of pregnancy following mating in the pacing chamber. While 50% of the female offspring of high-LG mothers became pregnant, over 80% of those of low-LG mothers were pregnant. These results suggest that the differences in sexual behaviour between the offspring of high- and low-LG mothers are indeed functionally relevant for reproductive success.

These findings reveal evidence for the maternal programming of sexual behaviour in the female rat. Moreover, maternal care is associated with alterations not only in sexual behaviour in the adult rat, but also in the timing of the onset of sexual behaviour. The female offspring of low-LG mothers show vaginal opening (an unambiguous indication of pubertal development in the rat) significantly earlier in life than do the offspring of high-LG dams. These findings provide a stunning parallel to the human literature (68) in which the onsets of both reproductive function and sexual activity are influenced by parental care in early life.

Perinatal 'maternal effects'

Cross-fostering studies with the newborn offspring of high- and low-LG mothers suggest maternal effects on the differentiation of female sexual behaviour that include 'maternal effects' during foetal development (57, 67). The sexual receptivity of the biological offspring of low-LG mothers reared by high-LG dams is indistinguishable from that of the normal offspring of high-LG mothers. These findings reveal a clear influence of postnatal maternal care. In contrast, the sexual receptivity of the biological offspring of high-LG mothers is unaffected by the rearing mother. This finding suggested a possible prenatal influence. Indeed, there are increased testosterone levels on embryonic day 20, a critical prenatal period for sexual differentiation in the rodent, in the female foetuses of high-LG mothers compared with those of low-LG mothers; the high-LG mothers themselves also show increased levels of testosterone. Exposure to elevated testosterone levels during foetal life results in a dampening of the pro-oestrous LH surge through a 'masculinisation' of the oestrogen positive-feedback effect on GnRH/LH systems (69). And there was additional evidence for foetal masculinisation (A. Del Corpo, N.M. Cameron and M.J. Meaney, unpublished data). Ovariectomised adult female offspring of high-LG mothers show increased male-like mounting towards a sexually receptive female by comparison with females reared by low-LG mothers. These findings suggest maternal effects on the differentiation of HPG function and female sexual behaviour that extend throughout the period of sexual differentiation in the rat.

Molecular mechanisms

To summarise, the offspring of low-LG mothers show evidence of increased sexual receptivity and decreased maternal LG. The offspring of high-LG mothers show precisely the opposite profile. And these differences in reproductive behaviours map onto those in ERα expression. These finding suggest that maternal effects on reproductive behaviour are mediated by tissue-specific differences in ERα expression in brain regions that regulate maternal and sexual behaviours. The remarkable feature of this effect is that the same stimulus input (maternal care) appears to regulate the expression of the same gene, the ERα gene, in exactly the opposite manner depending upon brain region (MPOA versus AVPVn).

A critical issue concerns the mechanism for the apparent maternal effect on ERα expression. Previous studies suggest maternal effects on the methylation status of promoters for steroid receptor genes in the rat brain (70–73). DNA methylation is associated with the repression of gene expression. This relation is mediated in part by effects on chromatin structure and the accessibility to transcrip-

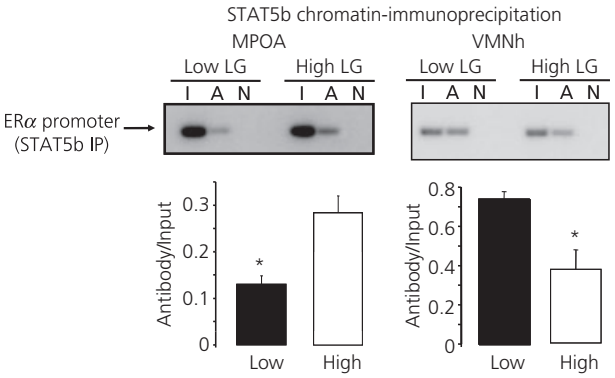

Fig. 3. The results of chromatin-immunoprecipitation assays examining signal transducers and activators of transcription protein 5b (stat5b) association with the exon 1b oestrogen receptor α promoter in samples derived from the medial preoptic area (MPOA) or the ventromedial nucleus of the hypothalamus (VMNh) from the virgin, adult female offspring of high- or low- licking/grooming (LG) mothers (n = 3–5/group). The upper panels are representative Southern blots showing total exon 1b oestrogen receptor α promoter abundance (Input; I), exon 1b oestrogen receptor α promoter was immunoprecipitated using the stat5 antibody (Antibody; A) or the neutral sequence control (N). Results are expressed as the mean ± standard error of the mean (SEM) ratio of antibody-precipitated/total (*P < 0.05).

tion factor binding sites (74–75). Methylated cytosines attract repressor complexes containing histone deacetylases that block the acetylation of histone tails, thus favouring a chromatin configuration that excludes transcription factor binding. We found evidence for significantly increased methylation across the exon 1b ERα promoter in the MPOA from the adult female offspring of low-LG mothers compared with those of high-LG mothers (49). ERα gene expression is commonly regulated by the activity of a JAK-Stat pathway (75), and chromatin-immunoprecipitation assays confirmed decreased Stat5 binding to the exon 1b promoter in the MPOA of low-LG mothers compared with high-LG mothers. Preliminary studies suggest the opposite pattern in the VMNh (Fig. 3): increased exon 1b promoter methylation and decreased Stat5 binding in VMNh samples from high-LG mothers compared with those from low-LG mothers. While it remains to be demonstrated that the methylation pattern is causally related to differences in ERα expression, these findings do reflect the remarkable capacity for tissue-specific regulation of the epigenetic status of common sites on the genome.

Conclusion

Reproduction represents a series of energy investments in mating and parental care. The *r* and *K* reproductive strategies in the female represent differential investment in mating versus parental care. The *r* strategy emphasises a strategy that maximises the *quantity* of offspring. In contrast, the *K* strategy emphasises the *quality* of offspring. The former involves increased investment in mating, the latter in parental care. While originally conceived as descriptions of species differences, more recent studies focusing on phenotypic variation within species reveal that both strategies can be represented within the same species, depending upon the prevailing environmental conditions. Indeed, it is reasonable to consider the *r* and *K* approaches as lying along a continuum and that the point along this continuum is, in certain species, defined by the quality of the environment and parental investment. Both strategies may be successful depending upon the prevailing environmental conditions. In some species, and this may include humans, there is evidence for a stable influence of environmental factors on reproductive phenotypes. Hence the same environmental conditions that compromise the early growth of the offspring and that 'programme' enhanced defensive responses may also alter the development of reproductive strategies (Fig. 1). The findings with the rat suggest that maternal behaviour may programme variations in reproductive strategies through tissue-specific effects on gene expression.

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