

Genomic Imprinting, Maternal Care, and Brain Evolution

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GENOMIC IMPRINTING

The majority of autosomal genes are inherited as two copies, one from each parent, and such biallelically inherited genes have identical functions. Genomic imprinting confers different functions on the two parental genomes during development by silencing one allele of each imprinted gene in a parent-of-origin-dependent manner. Hence imprinted genes usually function when inherited either from the mother or from the father, but, unlike sex-linked genes, are expressed in both sons and daughters. Imprinted genes are not expressed along the lines of classic Mendelian inheritance since the expression of an imprinted allele is dependent on whether it resided in a male or a female in the previous generation.

The transcriptional expression of imprinted genes is brought about by epigenetic modifications that are erased and reestablished during germ cell development. Methylation of the dinucleotide CpG has been extensively studied as a candidate marker for imprinting because of its association with transcriptional repression (Brenton *et al.*, 1999). The biological consequences of methylation are mediated by a family of methyl-CpG binding proteins, the best characterized of which is MeCP2, a transcriptional repressor that recruits histone diacetylases (Ng *et al.*, 1999; Hendrich *et al.*, 1999). The molecular mechanisms of genomic imprinting, nuclear reprogramming on passage through the germline, and the resistance to genomewide demethylation that occurs during preimplantation development are currently under intensive investigation

(Brenton *et al.*, 1999; Ben-Porath and Cedar, 2000; Reik and Murrell, 2000; Saitoh *et al.*, 1996).

The realization that male and female autosomal genes do not contribute equally to mammalian development derives from experiments in mice where normal development was found to require the presence of both a maternal and paternal genome (Surani *et al.*, 1984; McGrath and Solter, 1984). In these experiments both parthenogenetic embryos (diploid with two sets of maternal chromosomes) and androgenetic embryos failed to show normal development, and survival extended for only a few days beyond implantation. Parthenogenetic conceptuses developed embryos up to the 25-somite stage but the extraembryonic tissues which form the placenta were rudimentary. Androgenetic conceptuses possessed overdeveloped placental tissue, but the embryonic development rarely progressed beyond the 6- to 8-somite stage. Hence placental development came strongly under the influence of the expression of paternally inherited alleles, while embryonic growth was influenced by alleles of maternal origin, and these differing phenotypes resulted from the disruption of parent-of-origin-imprinted genes. These early observations have been substantiated by the identification of certain imprinted genes and by producing null mutations that experimentally produce some features of the placental/embryonic phenotype (De Chiara *et al.*, 1991).

EVOLUTION OF GENOMIC IMPRINTING

The evolutionary consequences and impact of genomic imprinting on mammalian development does not in itself explain why such a mechanism is required. A wide variety of theories have been advo-

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cated for genomic imprinting (Hurst, 1997), including conflict over parental investment (Moore and Haig, 1991), a defence mechanism [defence against parthenogenesis (Varmuzza and Mann, 1994), invasive retroviral DNA (Barlow, 1993), and chromosome gain or loss (Thomas, 1995)], while other theories relate to the regulation of gene dosage (Solter, 1988).

No single theory succeeds in completely explaining genomic imprinting, but since relatively few imprinted genes or their functions are known, these theories tend to address ultimate causation (Haig and Trivers, 1995). From a mechanistic viewpoint genomic imprinting has much in common with allelic exclusion in that only a single allele is expressed to encode RNA and protein, but in allelic exclusion the parent of origin for the allele is stochastic. The monoallelic expression ensures that only a single receptor type is expressed from a family of receptors and is exemplified in phylogenetically old systems such as the olfactory receptor genes, immunoglobulin genes, natural killer genes, and T-cell receptor genes (Chess, 1998). Such allelic exclusion has enabled expansion of receptor gene families while maintaining each cell's specificity at the same time. Likewise, expansion of gene control mechanisms has required cooperative binding of transcription factors regulated by a variety of signaling pathways. When a number of these factors must be present for any of them to bind, this may lead to all-or-none transcription, as is the case for the interleukin-2 (IL-2) gene, which has been recently shown to exhibit monoallelic expression (Hollander *et al.*, 1998). Dosage regulation for IL-2 is crucial since overproduction results in suppression of T-cell function and autoimmunity, while immunodeficiency occurs with decreased production. IL-2 expression is therefore tightly controlled by multiple signaling pathways. It is not just the cytokines like IL-2 which regulate biological effects in a dose-dependent mode, since during development localized gradients of chemokines specify cellular migration and growth factors are regulated according to a discriminating threshold. These events would also benefit from allelic exclusion.

Genomic imprinting is but a step down the road from allelic exclusion in that haploid regulation is determined by the parent of origin. Perhaps one of the first places where this occurred was in the X chromosome where the Xist gene (which determines X inactivation) became imprinted and paternally expressed in the extraembryonic membranes that form the placenta in eutherian mammals (Zuccotti and Monk, 1995). In marsupials the X chromosome shows im-

printed inactivation in several embryonic tissues and may have represented the ancestral form (Cooper *et al.*, 1993). It has recently been suggested that natural selection caused the establishment and maintenance of epigenetic differences between the maternal and paternal genomes of sexually reproducing organisms for the purpose of homologous pairing of chromosomes at meiosis and the associated process of DNA repair and recombination in meiosis and mitosis (Pardo-Manuel de Villena *et al.*, 2000).

PARENTAL CONFLICT

Whatever the explanation for the evolution of genomic imprinting, once in place it becomes subjected to differential selection pressures dependent on whether the gene is expressed through the matriline or patriline. Haig and colleagues (1991) have proposed that with the development of the placenta a conflict of interest between the maternal and paternal imprinted genes has arisen. Genetic evidence for this is seen with the paternally expressed *Igf2* (insulinlike growth factor 2) gene and the maternally expressed *Igf2* receptor, which binds the ligand but fails to transduce the signal to the cell (Haig and Graham, 1991). *Igf2* is known to promote placental growth and targeted mutations result in diminished growth of placenta and conceptus, while targeted mutation of the *Igf2* receptor promotes placental and, in turn, fetal growth. This theory of parental conflict in the context of genomic imprinting provided a conceptual framework that explains paternally expressed genes enhancing placental growth and maternally expressed genes restricting this growth. Since the mother provides the placental nourishment, it is in her interest to ensure survival of all her generations of offspring, while the interest of each father is to maximize the extraction of maternal resources for his own offspring at the expense of those from future fathers. One prediction of this theory is that genomic imprinting exists in species in which there is at least some contribution of maternal resources to the embryo and in which polyandry is observed. Comparative studies support this model since the *Igf2* gene is paternally expressed in the marsupial opossum, which, like eutherian mothers, support their offspring *in utero* via a primitive placenta derived from the yolk sac (O'Neill *et al.*, 2000). In contrast, *Igf2* is biallelic in birds, but since maternal allocation of resources to offspring in birds is completed prior to fertilization, the paternal genome has

no opportunity to affect the amount of resources the offspring receives (O'Neill *et al.*, 2000). Likewise, the *Igf2* receptor is imprinted in the marsupial opossum but not in the monotremes, where it does not bind *Igf2* and where a selective advantage to the paternal germline would not be provided by imprinting (Killian *et al.*, 2000).

A further two imprinted genes that are paternally expressed have recently been studied using gene targeting in embryonic stem cells. *Peg1* (also known as *Mest*) and *Peg3* are each imprinted and expressed only from the paternal allele. Both male and female *Peg1* offspring from normal mothers (i.e., inheriting the mutated allele from $+/-$ fathers) are smaller and weigh less than wild-type littermates (i.e., inheriting the normal allele from fathers) (Lefebvre *et al.*, 1998). Mutant embryos and placental weights are approximately 87% of normal and their postnatal growth is slower (65% of normal) in the first weeks postpartum. As adult animals they are still only 62% of the weight of normal littermates. Moreover, their survival rate is lower, with less than 50% of the expected number of mutant animals surviving to weaning. This finding, that the paternally expressed *Peg1 Mest* gene acts as a positive regulator of embryonic growth, is consistent with Haig's evolutionary model of genomic imprinting based on the conflicting interests of the parental genomes in mammals. The combination of mutant offspring born to a mutant mother was nonviable and not studied in detail since less than 5% of pups survived.

The second imprinted gene, *Peg3*, is also paternally expressed in embryonic placental tissues (Li *et al.*, 1999). To study the function of *Peg3* "in vivo," a targeted mutation of this gene was achieved by insertion of a β -geo selection cassette in the 5' coding exon. *Peg3* is expressed in both the fetus and placental trophoderm (Relaix, 1996) and although there are no obvious structural abnormalities, growth of both fetus and placenta appear affected in a systematic manner, with the placenta being more severely affected. At termination of pregnancy (day 18) the size of the placenta is reduced by 25% in embryos inheriting the mutation from father compared with maternal inheritance of the mutant allele. A close correlation between placental size and fetal growth in late gestation has been observed in humans and sheep (Robinson *et al.*, 1995) but the extent to which these are causally related is not clear. Prenatal mortality is also higher from normal mothers that carry offspring expressing the *Peg3* mutation, and those offspring born alive weigh signifi-

cantly less than those in wild-type litters. Postnatal growth of *Peg3* mutants throughout nurturing is also severely retarded even when reared by normal mothers, further suggesting either suckling impairment or endocrine growth hormone impairment. Since pituitary sommatotrophs show normal immunostaining for growth hormone and pup weight gain is normal postweaning, infant suckling ability is again probably the main cause of postnatal growth impairment.

A question of some relevance to our understanding of maternalism is whether Haig's theory can be applied to maternal behavior. The majority of paternally expressed imprinted genes so far cloned and sequenced are expressed in the placenta, and the placenta initially takes control of the mother's endocrine system to sustain pregnancy until the fetoplacental unit is self-sustaining. The mammalian placenta has developed the capacity to compress the endocrine function of several endocrine organs into one functional unit (Hardwenger *et al.*, 1994). Since these placental hormones stimulate maternal appetite and prime the brain to ensure that maternal behavior occurs contingent on parturition there is strong rationale for assessing how paternally expressed genes might impact on maternalism.

PATERNALLY EXPRESSED GENES THAT INFLUENCE MATERNAL BEHAVIOR

Peg1 gene in adult animals is expressed almost exclusively in the CNS (Lefebvre *et al.*, 1998). Particularly high levels of expression are found in the ventral forebrain, including the hypothalamus, amygdala, ventral hippocampus, and also in the main and accessory olfactory bulbs. Females carrying the targeted mutation have a normal pregnancy rate and deliver at term, but have few progeny which survive postnatally. Because the mutation is transmitted on the paternal allele, all of the pups are normal for the *Peg1/Mest* allele when the mutant mother has mated with a normal wild-type male. When cross-fostered to wild-type mothers, progeny from these matings show the normal rate of survival. Hence the low survival rate observed from mutant mothers is not attributable to a phenotype of their pups, but reflects a decreased maternal fitness of the mothers. Investigation of maternal behavior shortly after birth revealed that mutant *Peg1* mothers did not retrieve their pups, failed to suckle

them, and did not ingest the placentae. Furthermore, pup-induced maternal behavior in nonparturient females also revealed severe deficits in all aspects of maternal behavior in mutants (retrieval, nest building, and crouching) compared with wild-type females (Lefebvre, 1998). Sniffing of pups and location of buried food was not significantly different from wild-type females, suggesting the impaired maternal behavior was not primarily due to gross olfactory dysfunction.

Likewise, for the *Peg3* gene knockout mouse observations of maternal behavior with mutant primiparous mothers revealed a complete deficit in all aspects of maternal behavior (retrieving, nest building, and crouching). To validate these observations, the response of postpartum females toward cross-fostered newborn pups was observed during a 30-min period. Mutant mothers took 11 times longer to retrieve and 8 times longer to build a nest than wild-type females and were never observed to crouch over these pups. An inability to find the pups was not a factor in the impaired maternal response, as the mutant mothers sniffed the pups soon after introduction and as quickly as wild-type mothers. Further observations on nonparturient multiparous and virgin females for induced maternal behavior by pup exposure revealed significant impairments in all aspects of maternal responding compared with matched wild-type control females. Hence the mutation can affect maternal behavior independently of the hormonal influences of pregnancy and parturition, although these cannot be ruled out as contributing to the maternal phenotype when the developing fetoplacental unit inherited the mutation from the father.

Despite the small litter size of *Peg3*-mutant females, the surviving (nonmutant) progeny gain less weight during the first 3 weeks postpartum, although their weight did catch up after weaning. The preweaning deficiency in weight gain could result from impaired maternal response and/or a deficit in lactation in the mutant females. To test this we measured both maternal behavior and the weight gained by the pups following a 2-h separation from mother. The postpartum experienced mutant mothers were slower in adopting the crouching posture compared to wild-type mothers, but the pups of both groups attached to the nipples after 1 h. Pups' weight increased by 1.8 ± 0.5 and 3.2 ± 0.25 mg after 6 and 24 h, respectively, with normal mothers. By contrast, the pups suckled by the mutant mothers gained no weight after 6 h and only 0.98 ± 0.2 mg after 24 h. The reduced weight gain in

the latter suggested a defect in lactation in the mutant mothers (Li *et al.*, 1999).

Examination of the mammary glands of the mutant mothers showed them to be histologically normal both at prepartum and postpartum. Milk ejection is controlled by oxytocin released from the hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei in response to the suckling stimulus. Postpartum mutant mothers were found to have reduced oxytocin-positive neurons compared to the wild-type females. There were a total of 2984 ± 209 oxytocin-positive neurones in the mutant hypothalamus compared to 4496 ± 252 in the wild-type hypothalamus. Even allowing for the difference in body weight and PVN size between controls and mutant females, the number of oxytocin neurons was still significantly smaller in the latter. The reduced weight gain of pups could therefore be explained by an insufficient oxytocin surge, since a mutation of oxytocin gene in mice also abolished milk ejection.

Studies of neural mechanism of maternal behavior provides insight as to how *Peg3* may affect this behavior. A maternal response, elicited after a few days of pup contact, is protein-synthesis-dependent. The hypothalamic MPOA is known as a regulatory center for maternal behavior, as it probably integrates the female's response to multisensory stimulation from contact with pups, contains receptors where estradiol binds to promote maternal responsiveness, and shows *Fos* induction when females behave maternally or virgin females are exposed to pups (Calamadre and Keverne, 1994; Brown *et al.*, 1996). These transcription factors (*Fos* proteins or estradiol and its receptor complex) then activate the protein synthesis cascades required for the expression of maternal behavior. A similar role may be played by *Peg3*, presumably encoding a transcription factor and highly expressed in the MPOA. Given its potential capability of interaction with other proteins, it is possible that the *Peg3* gene functions as a cofactor of the neural substrates or transcription complexes described above, which are themselves modulated during induction of maternal behavior. Moreover, studies in rats have identified a neural circuitry whereby MPOA neurons have lateral efferent projections which signal to the brainstem structures involved in the expression of retrieval and crouching behavior (Numan, 1994). Colocalization of strong *Peg3* expression to parts of this neural circuitry provides a possibility that *Peg3* might participate in the processing of signal transduction to modulate the maternal response.

Recently, Brown *et al.* showed that *FosB* deficiency disrupts maternal behavior of both the postpartum and virgin females (Brown *et al.*, 1996), like the *Peg3* mutation presented here. However, pup-contact-directed *FosB* induction appeared to be unaffected by the *Peg3* mutation in our studies. Several possibilities could explain this observation: (1) *Peg3* functions independently of *FosB* in regulating maternal behavior; (2) it interacts with *FosB* but, in its absence, other proteins can compensate its function; and (3) it acts in the cascade downstream of *FosB* function in maternal behavior. Regarding the latter, it is of interest to note that the 5' flanking sequences of *Peg3* harbor several AP1 binding sites. Investigation of *Peg3* expression in *FosB* mutant mice should help to address this possibility.

In rodents, central oxytocin synthesis increases at parturition (Caldwell *et al.*, 1987), and central infusions of this hormone stimulate a rapid onset of maternal behavior (Pedersen *et al.*, 1982), while the hormone antiserum (Fahrbach *et al.*, 1985) or antagonists inhibits maternal behavior (Fahrbach *et al.*, 1985). Thus, the behavioral and neuroendocrine responses in this study have oxytocin as a common component for both the peripheral and central events that are impaired in the *Peg3* mutants. The reduced number of oxytocin-producing neurones would not eliminate oxytocin secretion but may have impaired the neuronal coupling and synchrony, which is required for a bolus of oxytocin release at postpartum to achieve milk letdown (Hatton *et al.*, 1987). The involvement of the *Peg3* protein in the TNF signaling pathway affecting NF κ B phosphorylation, apoptosis, and cell survival (Relaix *et al.*, 1998, 2000) could account for the developmental decreases in oxytocin neurons in the PVN and other hypothalamic neurones seen in *Peg3* mutants.

PARENTAL GENETIC CONFLICT (*Peg1* AND *Peg3*)

The observations that the mutant mice were smaller and the mothers had placental defects established that *Peg3* and *Peg1* act on the fetoplacental unit to promote growth and indirectly influence the nurturing ability of the female by priming the brain and mammary gland, thereby ensuring the survival of her offspring. These functions of paternally expressed genes are consistent with the theory of parental genetic conflict.

Haig and colleagues (1991, 1995) proposed that imprinting has evolved in mammals because of the conflicting interests of paternal and maternal genes within offspring. One such conflict is the maternal resources provided to offspring during intrauterine growth and the neonatal feeding period. In order to propagate themselves more successfully, the paternal genes are programmed to obtain maximum resources from the mother, even at the expense of future offspring, which may have different fathers. By contrast, the maternal genes would benefit by reducing the resource demand since they are present at a 50% probability in each of the other sibs. This model predicts that (1) imprinting will occur at loci which influence embryonic and placental growth, sucking behavior, appetite, nutrient metabolism, and postnatal growth rate; and (2) the paternal genes will promote growth, whereas the maternal genes will act to counter this effect.

The impaired behavior of the mutant mother with respect to paternally expressed gene deletion does not, from the evolutionary viewpoint concerning males, occur until the following generation, i.e., the mating males genes would only ensure his daughter showed good maternal care. Since this deficit would affect transmission of both grand paternal and grand maternal genes equally, it does not fit appropriately with the conflict theory. However, fixation of the gene in the population may have been boosted by the evolutionary advantage of its effects on maternal behavior, milk letdown, and postnatal growth. A second effect of these imprinted genes is on the offspring independent of the effects on mother and occurs when the mutant male is mated with a wild-type female and hence produces mutant offspring. Here we also see impairment in mutant female maternalism, with a reduced ability to sustain her pups. These effects can be considered secondary to the influence of placental hormones on the mother. Hence, imprinted genes which are paternally expressed orchestrate the provision of maternal resources both prenatally and postnatally by regulating the complementarity of behavioral and physiological events.

GENOMIC IMPRINTING, BRAIN DEVELOPMENT, AND BEHAVIOR

In early mammals the brain and the behavior it generated could be viewed as making an integrative contribution to the animals physiological homeostasis

and a means of providing early mammals with information about their habitat and social environment. The special senses provided information on both the physical and social environment, while the colonization of more temperate zones selected for an ability, in the brain, to predict the changing environment by measuring subtle changes in daily photoperiod. Seasonal breeding assured that birth and weaning of offspring occurred at times optimal for survival and food availability. These early mammalian brains also needed to integrate information from the internal environment in order to generate behavior that sustained homeostasis. In such small-brained mammals, most motivated behaviors could be viewed as part of behavioral homeostasis. Hence feeding was stimulated by hunger, sexual behavior was determined by gonadal hormones, and maternal behavior was dependent on the hormones of pregnancy generated by the fetoplacental unit. These generalizations still apply to most small-brained mammals, but in mammals with larger brains such as monkeys, apes, and humans that exhibit complex social organization, such behavior is not regulated in any simplistic way by either endocrine or genetic determinism.

In considering primate maternal behavior, strong interrelations are beginning to emerge between the two important features, neocortical expansion and matrilineal inheritance. These features are common to most mammals that exhibit a complex social organization (Gittleman, 1994) but are especially prevalent in simian primates (Dunbar, 1992). The development of a larger neocortex has enabled motivated behavior to occur at will such that maternal affiliation may take place without pregnancy and parturition (Keverne *et al.*, 1996). This unique development in human evolution has matched parturient females with nonparturient females in sustaining the behavioral potential for infant caregiving. Decreasing the dependence of maternal behavior on endocrine determinants has been an evolutionary necessity in order for infant care to extend long beyond weaning and hence beyond the influence of pregnancy or suckling hormones. In most mammals, maternal care ceases when suckling terminates. Progression away from the synchronization of maternal behavior with the hormones of pregnancy and increasing dependence on cognitive control requires exceptional cognitive abilities. This became evolutionarily viable with the development of a large neocortex since the decision-making processes in the context of maternal behavior are complex and need to be strategically correct. Such abilities are not inherited,

but require learning and the kind of experience that a large brain provides. This would explain the overriding importance of social and maternal experience in order to achieve successful maternal care (Holman and Goy, 1995; Kraemer, 1991; Berman, 1990), experience that is acquired during early social development under the watchful eye of mothers, but outside the immediate context of pregnancy and parturition (Keverne, 1995).

From the available fossil records it appears that many mammalian lineages have evolved increased cranial capacity (Armstrong, 1982), but because it is claimed that the push for an exceptionally larger neocortex in primates has developed from complex social living (Dunbar, 1992), then differences in maternal and paternal lifestyles may have subjected brain evolution to differential selection pressures. In Old World monkeys, females provide social stability and group cohesion, are more affiliative than males, and maintain the continuity of the group over successive generations (Wrangham, 1980). Females are the primary caregivers, with social rank of daughters, but not sons, being related to the matriline (Smuts *et al.*, 1987). This kind of matrilineal inheritance is compatible with genomic imprinting (Keverne *et al.*, 1997).

Investigating genomic imprinting in the brain could theoretically be achieved by constructing parthenogenetic embryos where both sets of alleles are maternal in origin. Autosomal alleles that are inherited in a normal Mendelian way would be unaffected in parthenogenomes, but imprinted genes that are only expressed when inherited from mother would be duplicated. Unfortunately, parthenogenetic and also androgenetic embryos die at the earliest stages of development, long before the brain starts to develop (Surani *et al.*, 1990). However, investigating genomic imprinting in the brain has been achieved by the construction of chimeras made up of parthenogenetic cells (containing *LacZ* genetic marker) and normal cells (Keverne *et al.*, 1996). These chimeras are not embryonic lethal so long as the parthenogenetic cells do not constitute more than 40% of the embryo. In this way, the normal cells rescue the developing embryo from lethality while the cells disomic for maternal alleles compete with normal cells and proliferate in those areas where having two sets of maternal alleles but no paternal alleles are developmentally advantageous. Using these techniques, a clear and distinct patterning in the distribution of chimeric cells emerges during brain development. At birth, cells that are disomic for maternal genome (i.e., both alleles are from mother)

contribute substantially to neocortex, striatum, and hippocampus but are excluded from those parts of the brain that are important for primary motivated behavior (hypothalamus, septum, preoptic area, and BNST) (Allen *et al.*, 1995). At the earliest stages of brain development (days 9 and 10) parthenogenetic cells are not seen in the basal forebrain plate, while in contrast, androgenetic cells (disomic for paternal genome) are present in all neural tissues and as brain development proceeds they proliferate in mediobasal forebrain and at parturition are virtually absent in telencephalic structures. Furthermore, growth of the brain of parthenogenetic chimeras is enhanced by this increased maternally expressed gene dosage, whereas the brains of androgenetic chimeras are smaller, both in absolute size and especially relative to body weight (Keverne *et al.*, 1996).

Not only is it surprising that parthenogenetic cells seem to proliferate at the expense of normal cells and to produce a larger telencephalon in chimeras, but this enlarged brain appears anatomically and functionally normal. This is surprising because a large number of genes have been silenced in these cells (i.e., all the imprinted genes that are paternally expressed), and others that are maternally expressed have been duplicated. This would seem to emphasize the importance of maternally expressed alleles in telencephalic development and the lack of importance of paternally inherited genes in these regions. The distinct patterning in the distribution of parthenogenetic and androgenetic cells and their differential effects on brain growth suggest genomic imprinting may have been important in forebrain evolution. Allometric scaling of the parts of the brain to which maternally or paternally expressed genes differentially contribute reveals that a remodeling of the brain has occurred during mammalian evolution. On moving across phylogenies from insectivorous mammals to prosimian and then simian primates, these allometric studies show that the neocortex and striatum have increased significantly in size relative to the rest of the brain and body, while the hypothalamus, medial preoptic area, and septum have decreased in size (Keverne *et al.*, 1996). Genomic imprinting may thus have facilitated a rapid, nonlinear expansion of the brain (especially the neocortex and striatum) relative to body size during its development over an evolutionary time scale.

The progressive emancipation of behavior from hormonal control has required a parallel evolutionary development of an "executive" brain that is capable of getting decisions right. Wrong decisions in the context

of sexual and parental behavior could be very costly for sustaining reproductive success. Genetic imprinting is, therefore, perfectly compatible, with the two genomes cooperating to produce viable whole-brain function. It is certainly the case that when the balance of the two imprinted genomes is disturbed in certain human genetic disorders, brain dysfunction occurs. Interestingly, this is characterized by mental retardation, movement disorders, and speech difficulties [maternally active alleles deleted; Wagstaff *et al.*, 1992] (cortex and striatum) or overeating and sexual dysfunction [paternally active alleles deleted; Christian *et al.*, 1995; Nicholls, 2000] (hypothalamus and medial preoptic areas)].

The behavioral interactions of female Cercopithecine monkeys relate to the social hierarchy in a way that is very different from males (Keverne, 1993); so the way in which the brain is used can clearly differ between the sexes. Although the brain functions as a unitary structure, its evolution may have benefited from differential selection pressures on its constituent parts created by the differences in lifestyle which are best suited to the reproductive success of each sex. The further emancipation of sexual and maternal behavior from gonadal hormone control has enabled this kind of behavior to occur at will such that maternal affiliation can occur without pregnancy and parturition, and sexual activity is not confined to short, discrete periods of estrus. This unique development in human evolution has matched female and male in a continual readiness for sexual activity and parturient females with nonparturient females in maintaining the behavioral potential for infant caregiving. Both events must have a large impact on the way societies evolved and both events have benefited from the differential development of brain structures controlled by imprinted genes.

CONCLUSIONS

A complete understanding of any behavior requires a multidisciplinary approach and a need to cross and recross the boundaries between different levels of complexity. Maternal behavior is no exception and although this article focused on genomic imprinting, this in turn provides a key to unlocking the complexities of brain evolution and brain development, both of which are crucial to understanding the differences in the way neuroendocrine mechanisms regulate maternal behavior.

Genomic imprinting is, among the vertebrates, unique to mammals and imprinted genes appear to be regulatory genes, found in clusters on a number of different chromosomes. It is perhaps too early to be categorical, but imprinted genes appear to be conserved and are not rapidly evolving and therefore must have had their evolutionary impact on development by regulating other genes and complex cellular signaling pathways. In this context it has become clear that an understanding of maternalism in mammals needs to go beyond maternal behavior, taking into account feeding, placentation, body temperature regulation, lactation, and rates of infant growth and brain development. Although there are many genes which impact on maternal behavior (*FosB*, dopamine β -hydroxylase, *Mest*, *Peg3*, etc.) maternal behavior would fail unless it were integrated with other aspects of maternalism. It is therefore most unlikely that maternal behavior evolved in isolation from these other aspects of maternalism. What we observe from the studies of targeted mutagenesis of the paternally expressed genes (*Peg1* and *Peg3*) are multiple actions at two different levels. Both genes regulate fetoplacental growth and development, which is inclusive to the "genomic conflict theory" and both act directly on the mother to regulate maternal behavior, which was probably important for establishment of the gene in the population. Interestingly, these imprinted genes are complimentary by their actions in the fetus and the mother, e.g., suckling and milk letdown, brain priming by placental hormones for induction of feeding, and maternal behavior. There is also complementarity within the mother, e.g., maternal thermogenesis and nest-building behavior. Hence, a single imprinted gene which is paternally expressed can orchestrate the genetic cascade which provides for maternal resources both prenatally and postnatally by regulating the complementarity of behavioral and physiological events.

Genomic imprinting has also been important in remodeling of the brain as exemplified by the changes that have occurred in relative size of executive and hypothalamic structures across mammalian phylogenies on moving from insectivores to prosimian to simian primates. The vast enlargement of the neocortex with corresponding contraction of the hypothalamus has been significant in the move to greater cognitive control over behavior. Enlargement of the brain beyond the capacity of the birth canal has been accomplished by delaying much of its development to the postnatal period. Such neotamous growth of the brain can take years to complete in simian primates and

especially humans, which in turn requires prolonged offspring care. This is best accomplished by living in social groups, enabling kin to both assist in parental care and gain experience of caregiving, a valuable strategy for their future maternal success with the emancipation from endocrine determinism. So why genomic imprinting? Whenever there are asymmetries in the expression of imprinted alleles then they can be subject to differential selection pressures. Living socially has certainly been a selection pressure for the development of intelligent behavioral strategies. However, in large-brained simian societies it is the matriline that sustains social stability and continuity, with males leaving the group. Hence if living socially requires intelligent behavioral strategies that benefit from a larger telencephalon, then asymmetries for selection pressures favor the matriline and maternally expressed alleles.

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