What Are Imprinted Genes Doing in the Brain?

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Abstract

As evidence for the existence of brain-expressed imprinted genes accumulates, we need to address exactly what they are doing in this tissue, especially in terms of organisational themes and the major challenges posed by reconciling imprinted gene action in brain with current evolutionary theories attempting to explain the origin and maintenance of genomic imprinting. We are at the beginning of this endeavor and much work remains to be done but already it is clear that imprinted genes have the potential to influence diverse behavioral processes via multiple brain mechanisms. There are also grounds to believe that imprinting may contribute to risk of mental and neurological disease. As well as being a source of basic information about imprinted genes in the brain (e.g., via the newly established website, www.bgg.cardiff.ac.uk/imprintedgenes/brain_table), we have used this chapter to identify and focus on a number of key questions. How are brain-expressed imprinted genes organised at the molecular and cellular levels? To what extent does imprinted action depend on neurodevelopmental mechanisms? Do imprinted gene effects interact with other epigenetic influences, especially early on in life? Are imprinted effects on adult behaviors adaptive or just epiphenomena? If they are adaptive, what areas of brain function and behavior might be sensitive to imprinted effects? These are big questions and, as shall become apparent, we need much more data, arising from interactions between behavioral neuroscientists, molecular biologists and evolutionary theorists, if we are to begin to answer them.

Imprinted Genes and the Brain

Imprinted genes, in contrast to most mammalian genes, are monoallelically expressed in a parent-of-origin dependent manner. Early evidence from human and animal studies suggested that they were likely to play a fundamental role in basic growth and development, but recent work has suggested that in some cases they may also mediate more subtle effects on ongoing physiological processes. Imprinted genes are expressed in a wide range of tissues, but are particularly highly expressed in the placenta and the brain. Although much work remains to be done, there is now convincing and convergent functional evidence from a variety of sources (summarized below) that imprinted genes play an important role in the development and/or the ongoing function of the brain and that imprinted gene dysfunction may predispose to several common neurological/neuropsychiatric disorders. Addressing the extent to which imprinted genes contribute to normal brain development and function and therefore the extent to which imprinted gene malfunction may be implicated in brain abnormalities, will constitute an important focus of research over the coming years. In order to facilitate these objectives, we have recently established a freely accessible...
and updatable database listing all brain-expressed imprinted genes, their expression patterns, their putative functions and information on their imprinting status in other tissues (www.bgg.cardiff.ac.uk/imprinted_tables/index.html).

Summary Evidence for a Role for Imprinted Genes in Brain Function

- Mouse models in which the dosage of imprinted genes has been reduced (e.g., by deletion of the gene) or enhanced (e.g., by uniparental disomy) commonly show brain and behavioral phenotypes (reviewed in ref. 7).
- Cells containing solely paternally or maternally inherited diploid genomes localize to different brain regions and appear to affect brain growth in mice (and see later).
- Some behavioral phenotypes in mice (e.g., urinary odour preference) may be subject to parent-of-origin effects consistent with the influence of underlying imprinted genes.9,10
- In humans, cytogenetic disruptions of imprinted gene-rich regions may be associated with aberrant neurobiology e.g., 15q11-q13 abnormalities lead to the neurobehavioral disorders Prader-Willi and Angelman syndromes (PWS and AS respectively),11 and maternal duplications of this region may also be related to autistic phenotypes.5
- Some linkage and association findings are sensitive to the parental origin of the region of interest, perhaps indicating the influence of underlying imprinted genes.9,10
- Certain neuropsychiatric and neurological disorders (e.g., Tourette’s syndrome, panic disorder, multiple sclerosis, Alzheimer’s disease, schizophrenia and bipolar disorder) appear to be preferentially transmitted from one parent, again potentially suggestive of underlying imprinted genes, though other mechanisms could also explain this bias.5

Characteristics of Brain-Expressed Imprinted Genes

Given our current limited state of knowledge of brain-expressed imprinted genes, it is difficult, at present, to identify any common themes uniting imprinted gene action. Certainly, a cursory look at the data available at the molecular level seems to indicate a multitude of functions, from neurotransmitter receptor subunits to proteins involved in chromatin modification.3 Furthermore, imprinting in the brain is a complex and spatiotemporally dynamic process (even more so than in other organs given the heterogeneity of the tissue) and these factors constitute a major challenge when examining imprinting at the cellular level. There is however, evidence consistent with the existence of some degree of patterning with respect to cell-type specific imprinting. Cell culture studies have revealed that murine Ube3a sense transcripts are maternally expressed in neurons and biallelically expressed in glia, whereas the paternally expressed antisense transcript, Ube3as, is expressed only in neurons.14 In contrast, Igf2r is preferentially maternally expressed (with its antisense transcript Air paternally expressed) in glial cell cultures, but in neurons Igf2r is biallelically expressed and Air is not expressed.15 The significance of this cell-specific imprinting remains to be determined, but it is certainly possible that, for some genes at least, there is some functional correlation between their imprinted status and their roles in different cell types.

In terms of their spatial expression patterns, most brain-expressed imprinted genes seem to be expressed and imprinted in at least one other organ; however, there are several exceptions to this rule. Studying these exceptions may give important insights into the precise role of imprinting in the brain. For example, the imprinted Nnat gene (encoding neuronatin) appears to be expressed in a relatively neural-specific manner,16 whilst the genes Zim1 (encoding a zinc finger protein)17 and Ppp1r9a (encoding neurabin) are imprinted in other tissues but biallelically expressed in the brain. Conversely, UBE3A is biallelically expressed everywhere but the brain,18 whilst its murine homologue Ube3a is biallelically expressed throughout most of the brain, but is imprinted in specific brain regions (olfactory bulb, hippocampus and Purkinje cells of the cerebellum);19 Igf2 may also only show imprinted expression in highly specific brain regions.20 In general, brain-expressed genes
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seem to be persistently imprinted (although overall expression levels of several imprinted genes including \(X_{rb}3b\), \(Ndn\), \(Mkrn3\) and \(Magel2\) (D. Relkovic, L.S. Wilkinson and A.R. Isles, unpublished data) decline markedly throughout development, perhaps consistent with them exerting their greatest effect during embryogenesis). However, for a number of these genes, imprinting has only been demonstrated at a single time-point, so the generality of this conclusion remains to be tested. There are also exceptions to this pattern of imprinted expression occurring predominantly during early development. \(Commd1/Murr1\) shows strong maternal expression in adult brain, but biallelic, or weak maternal expression in embryonic and neonatal brain.\(^22\)

Imprinted Gene Effects on Brain Development

Seminal mouse studies in the mid 1990s revealed that imprinted genes are likely to contribute significantly to brain development and also indicated potentially dissociable (and antagonistic) influences of paternally and maternally expressed genes on this process. Briefly, chimeric mice were created which contained either a mixture of androgenetic (Ag) (containing two paternal genomes but no maternal genome) and normal cells, or a mixture of parthenogenetic (Pg) (containing two maternal genomes but no paternal genome) and normal cells. Pg chimeras' displayed relatively large brain:body size ratios, whilst Ag chimeras' displayed relatively small brain:body size ratios, implying that one or more imprinted genes have profound effects on brain size.\(^3\) Specifically, the data seem to indicate that the overall effect of maternally expressed genes is to enhance brain size, whilst the combined effect of paternally expressed genes is to limit brain growth. More detailed neuroanatomical studies on the Ag and Pg chimeras will enable us to decipher why their brains differ in size (and hence which processes the underlying imprinted genes may be affecting). Increased brain size in Pg chimeras could theoretically be due to increased proliferation of neuronal (or glial) cells, an increase in neuronal (or glial) size perhaps related to aberrant pruning, or a decrease in cell death/apoptosis. Interestingly, the distribution of the Pg and Ag cells in the two types of chimera was reciprocal, with Pg cells contributing mainly to the neocortex and Ag cells contributing more to the hypothalamic, septal and pre-optic areas. Again, it is feasible that these effects represent either the combined effects of many paternally and maternally expressed imprinted genes, or the actions of one or two imprinted genes of major effect. If the former is the case, we may expect maternally expressed imprinted genes to be disproportionately expressed in neocortical regions and paternally expressed imprinted genes to be disproportionately expressed in hypothalamic and septal regions. However, based on our current knowledge this conclusion does not appear to be supported.\(^3\) For a further discussion of these chimeric mouse experiments, see the chapters by Goos and Ragsdale and by Frontera et al.

Further evidence for an important role of imprinted genes in neurodevelopment has come from studies of mutant mice and humans with imprinting disorders such as AS and PWS. Currently such studies are rare, but as the number of imprinted genes discovered and knockout mice created continues to rise (and as the significance of imprinted gene function to neurobiological processes begins to be realized) more data will accumulate. In mice, the deletion of \(Ndn\), a candidate gene for PWS, results in morphological abnormalities in axonal outgrowth and fasciculation in several regions of the nervous system during embryogenesis.\(^23\) Necdin-deficient mice exhibit a reduction in both oxytocin-producing and luteinizing hormone-releasing hormone (LHRH)-producing neurons in adult hypothalamus,\(^24\) augmented apoptosis in the sensory ganglia and a reduction in the numbers of substance-P containing neurons.\(^25\) These neuroanatomical abnormalities may explain the characteristic PWS behavioral profile (described later). Deletion of \(Peg3\) similarly results in reduced numbers of oxytocin-producing neurons in the hypothalamus of adult female mice.\(^26\) Deletion of the AS candidate gene \(Ube3a\) does not affect gross neuroanatomy but does lead to accumulation of cytoplasmic p53 and deficits in long term potentiation\(^27,28\), whilst abnormal cerebellar folding was observed in mice disomic for distal chromosome 2, including \(Nnat\).\(^29\) In some instances, such as in deletion of \(Gnasxl\), \(RasGrf1\) and \(Nesp\), no gross effects on brain morphology have been observed; however subtle effects on fundamental processes such as neuronal outgrowth in the case of \(RasGrf1\) knockouts\(^30\) and vesicular release in the case of \(Nesp\) knockouts\(^31\) cannot be
discounted. More comprehensive neuroanatomical studies using imprinted gene knockouts will enable us to identify such abnormalities.

With respect to human work, imaging studies in PWS subjects have revealed abnormalities in pituitary, cortical and brainstem development, whilst studies in AS subjects have shown cerebellar and Sylvian tissue abnormalities, with ventricular enlargement and hypoperfusion of some brain regions. With studies such as these, where subjects do not have highly discrete genetic lesions, caution must be applied during interpretation since any neuroanatomical phenotype could simply result from the aberrant expression of non-imprinted brain-expressed genes rather than from imprinted gene effects per se.

**Imprinted Gene Effects on Behavior**

Parallel work in humans and mice has suggested that imprinted genes may not only affect brain development but may also impact upon a wide range of behavioral phenotypes. Explicit imprinted conditions such as PWS and AS are associated with distinct behavioral profiles. PWS is characterised by early hypotonia followed by a compulsive desire to eat (probably reflecting impaired satiety mechanisms) and is associated with mild mental retardation, insensitivity to pain, tantrums, obsessive tendencies, skin picking, unusual skill with jigsaws and in some cases psychosis. AS is characterised by mental retardation, ataxia, what has been termed a ‘happy’ disposition and repetitive or stereotyped behaviors. These behavioral phenotypes imply that imprinted genes may affect both primary-motivated behaviors and higher-level cognitive functions; however, again there is the possibility of contributions from abnormal expression of non-imprinted brain-expressed genes. There is also other evidence for imprinted gene effects on behavior in man related to the sex chromosomes. For example, the behavioral profile of Turner’s syndrome (TS) subjects (who possess a single X chromosome) depends upon the parental origin of this chromosome, suggesting an influence of one or more, as yet unidentified, X-linked imprinted genes. TS subjects inheriting their X chromosome maternally (45,X<sup>m</sup>O) display impaired social cognition (specifically behavioral flexibility) and are significantly more vulnerable to autism than subjects inheriting their single X chromosome paternally (45,X<sup>p</sup>O); however, the former group demonstrate superior performance than the latter group on a task assaying visuospatial memory. As X-linked imprinted genes may be expressed in a sexually dimorphic manner and may influence sex-limited behavioral traits and sex-specific vulnerability to certain mental disorders, the identification of such genes in man is an important future goal. Clues as to the identity of human X-linked imprinted genes may be gained from the recent identification of novel X-linked imprinted genes in mice. Other aspects of the phenotypes associated with AS, PWS and TS are discussed in the chapters by Frontera et al and by Goos and Ragsdale.

In mice, the first behavioral phenotype to be associated with imprinted gene activity was observed in neonatal mice disomic for chromosome 2; mice with paternal uniparental disomy appeared hyperkinetic whilst mice with maternal uniparental disomy were hypokinetic. Since that study, more sophisticated behavioral analyses have been performed on a number of imprinted gene knockout mice. Behavioral work so far has mainly been directed by our limited knowledge of the expression patterns and functions of the disrupted imprinted genes; this ascertainment bias means that only a small proportion of the repertoire of behaviors that imprinted genes influence is likely to have been uncovered as yet. Thus, it is still too early to say whether the deletion of paternally and maternally expressed genes leads to dissociable and opposite phenotypes, a key question. Ideally, a given mutant should undergo a comprehensive battery of behavioral tests assessing sensorimotor, ‘emotional’ and cognitive functions to reveal any interesting phenotypes not predicted a priori. Yet even now it is clear that imprinted genes may influence a wide range of murine behaviors, from primary motivated feeding behaviors to higher level cognitive processes. In neonates, deletion of the paternally expressed Gnasxl leads to impairments in suckling, consistent with expression of this gene in regions related to innervation of the tongue and jaw muscles. In adult mice, deletion of the paternally expressed genes Peg1/Mest and Peg3 is associated with impaired mothering; presumably a consequence of abnormal hypothalamic development. Deletion of Ube3a has re-
sulted in deficits of context-dependent memory\textsuperscript{27} whilst deletion of \textit{RasGrf1} has been shown to lead to impairments in memory consolidation\textsuperscript{44} and possibly long term depression.\textsuperscript{49} Deletion of \textit{Ndn} recapitulates some of the behavioral facies seen in PWS including improved spatial learning and memory and skin scraping.\textsuperscript{45} Finally, deletion of the maternally expressed \textit{Nesp} gene results in abnormal reactivity to novel environments in adult animals.\textsuperscript{46} Importantly, for several of these knockout mice, the behavioral effects in adults are independent of gross effects of the gene on development (see later).

**Through What Mechanisms Might Imprinted Genes Affect (Adult) Behavior?**

As many imprinted genes are expressed in the brain and placenta during embryogenesis and many remain expressed in adult brain, a number of possibilities exist with regard to how they may affect adult brain function (and hence how they may affect vulnerability to adult psychiatric disease when aberrantly expressed). The expression of some imprinted genes during neurodevelopment (e.g., \textit{Ndn}) may have direct and major, effects on neuronal growth and pruning, axonal sprouting and interconnections that take place during this critical period of time, with important consequences for brain functionality and connectivity. A recent bioinformatic screen in mice has predicted imprinting of two crucial genes in brain development, \textit{Bdnf} and \textit{Gdnf};\textsuperscript{47} whether these genes are actually imprinted in mice, (and/or humans), remains to be tested, but the functional ramifications of imprinting of these genes would be far-reaching.

However, several imprinted genes that are not expressed in brain may also theoretically affect neural function in an indirect manner. For example, imprinted genes such as \textit{Igf2} and \textit{Slc38a4} seem to have a major role in governing transfer of essential nutrients (e.g., glucose, amino acids) across the placental membranes,\textsuperscript{48} and too much or too little of these resources could lead to many downstream effects in the future, abnormal brain development being one of them. Moreover, these changes may not show directly in the offspring but may lead to an initially silent ‘programming’ that only becomes effective in adulthood. Such latent programmes may become activated in adulthood, e.g., as a result of a stressor, the idea behind Barker’s hypothesis; ‘the developmental origins of adult disease’.\textsuperscript{49} Conditions such as type-2 diabetes, hypertension and osteoporosis have been the major focus of such work but certain triggers may in some circumstances result in an increased vulnerability to mental and behavioral problems. Such effects may be particularly apparent if an individual, when born small (e.g., as a consequence of placental insufficiency), experiences a period of ‘catch-up’ growth.\textsuperscript{50,51} Imprinted genes may also influence adult phenotypes at another key developmental stage, the preweaning environment and there is at least one imprinted gene, \textit{RasGrf1}, that does not affect gross embryonic development (knockout mice were of equivalent weights to their wildtype littermates at birth), but that may affect perinatal growth.\textsuperscript{52} Therefore, in addition to affecting neurodevelopment directly (the gene is expressed throughout the brain) \textit{RasGrf1} may also affect brain development indirectly, via effects on preweaning growth and development. It is well established that early life mother-pup interactions can profoundly influence the development and function of the nervous system, as highlighted, for example, by studies examining the neurobiological sequelae of maternal deprivation in rodents.\textsuperscript{53} Thus, imprinted genes such as \textit{Peg1} and \textit{Peg3}, which influence behavior of mothers towards their offspring, may shape the future behavior of their offspring via this epigenetic route. Recent exciting work by Michael Meaney’s group, in which they showed that the epigenetic status of the glucocorticoid receptor and estrogen receptor genes and neuronal survivability in the hippocampus of pups depends upon the level of maternal care (specifically licking),\textsuperscript{54-56} has highlighted the possibility of an imprinted gene-dependent ‘loop’ i.e., imprinted genes may influence certain types of behavior (such as maternal licking and grooming), which in turn elicit effects on the expression of genes particularly sensitive to epigenetic perturbation in their offspring, potentially including imprinted genes themselves.

The ways in which imprinted genes affect brain development and function during embryogenesis and the perinatal period are, as can be appreciated from the above, likely to be complex and inter-dependent. Additionally, the fact that many imprinted genes are expressed into adulthood
implies that imprinted gene function may be directly relevant to adult brain function per se. The most convincing explanation for the evolutionary origins of imprinting, the conflict theory, posits that imprinting has arisen as a consequence of the differential interests of the two parents with regard to provisioning of their common offspring (see Chapter by Moore and Mills). A recent adaptation of the original conflict theory, the kinship theory, predicts that asymmetries of relatedness within a social group (e.g., when there is sex-biased dispersal) may provide a route by which intragenomic conflict impacting upon behavioral functioning could arise. This idea is important on two levels; firstly it suggests that imprinted genes may influence adult brain function; second it points to (in the broadest sense) the behavioral substrates that may be influenced, namely social interactions within the groups.

Imprinted Genes in the Adult Brain

Given the preponderance of data showing that many imprinted genes have gross effects on growth (whether prenatal or peri/postnatal), to what extent may adult imprinted brain functions be ascribed to epiphenomena? Is the primary (and adaptive) function of all adult brain expressed imprinted genes simply growth modulation? There is some, admittedly limited, data that address this issue. Firstly, many genes show continued imprinting in the adult brain and some, like Ube3a, show imprinting in specific and discrete regions of the brain. Given that monoallelic expression of a gene may be detrimental, insofar as the advantages of diploidy are effectively lost, this implies some degree of specific functionality in the imprinting status of a given gene persistently expressed in the adult brain. (See also the chapter by Ubeda and Wilkins.) Additionally, as mentioned previously, the gene Commd/Murr1 only appears to be fully imprinted in the adult brain. Thus, we might speculate that imprinting may not only be important for early life processes, but that it may also be functionally important for so-called ‘online’ adult brain functions. Altering the dosage of such a gene in mouse mutants, together with further investigations into its neural function will allow us to determine whether its imprinting in the adult is likely to be of any adaptive significance, or whether it is merely a redundant side-effect. Recently we, in collaboration with the group of Gavin Kelsey, have shown that the maternally expressed gene Nesp, part of the Gnas cluster, influences adult behavior. Mice carrying a maternally derived null allele of Nesp demonstrated altered reactivity to a novel environment as adults, with no obvious concomitant effects on fetal growth, placental function, early postnatal growth or survivability. This implies that Nesp, which is expressed in discrete regions of the adult brain, directly influences ongoing adult brain function, or elicits effects on adult brain function via subtle effects on neurodevelopment. However, this is not totally conclusive (for instance as discussed previously subtle effects on mother-pup interactions may give rise to large changes in the offspring when adult) and additional behavioral and neurobiological studies of this mouse, plus future work using a conditional Nesp knock-out, will address this issue more definitely.

What Adult Behaviors Will Imprinted Genes Influence?

One route through which imprinted genes could have evolved to influence adult brain function directly is where there is sex-biased dispersal from a social group and from this we can make a prediction that imprinted genes will influence behavior that impacts on social interactions. Asymmetries of relatedness will occur with either male or female biased dispersal, but the most common situation in mammals is for males to disperse upon reaching sexual maturity, producing “matrilineal” social groupings. If we take these as an example, given the greater sharing of maternal alleles between group members, we may expect these to promote social cohesion within the group. However, paternal alleles, which are less widely shared, will seek to limit any behavior that may reduce their presence in the next generation.

The range of behaviors that can be thought to be important to a social group is wide, taking in alarm calling, resource foraging and gathering, shared care of young and specific social cohesion behaviors such as grooming. For instance, as Nesp knockout mice show a reduced propensity to explore a novel environment, we could tentatively suggest that the function of this maternally
expressed gene is to promote foraging behavior. One putative neural system that may also be influenced by imprinted genes is that involved in communal care of offspring,\(^6^3\) such as the oxytocin system.\(^6^4\) Indeed there is some evidence that this is the case, in that Peg3 appears to be involved in development of oxytocin neurons and maternal behavior.\(^6^5\) More generally, imprinted genes may impinge on those systems involved in general affiliative behaviors, such as vasopressin.\(^6^6\) In humans, it is possible that brain processes and behaviors associated with higher level social communication (i.e., speech, reading and language) may be subject to imprinted gene dependent parent-of-origin effects.\(^6^7\) (For further discussion, see the chapter by Goos and Ragsdale.) By stratifying linkage data from disorders of sociality/language such as autism and dyslexia according to parental origin, we should eventually be able to identify and characterise imprinted genes affecting these important psychological constructs.

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