Frontiers in Neuroendocrinology 53 (2019) 100732

Contents lists available at ScienceDirect

Frontiers in Neuroendocrinology

journal homepage: www.elsevier.com/locate/yfrne

Review

Imprinted genes influencing the quality of maternal care

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ARTICLE INFO

Keywords:
Genomic imprinting
Maternal care
Placenta

ABSTRACT

In mammals successful rearing imposes a cost on later reproductive fitness specifically on the mother creating the potential for parental conflict. Loss of function of three imprinted genes in the dam results in deficits in maternal care suggesting that, like maternal nutrients, maternal care is a resource over which the parental genomes are in conflict. The induction of maternal care is a complex, highly regulated process and it is unsurprising that many gene disruptions and environmental adversities result in maternal care deficits. However, recent compelling evidence for a more purposeful imprinting phenomenon comes from observing alterations in the mother's behaviour when expression of the imprinted genes *Phlda2* and *Peg3* has been manipulated solely in the offspring. This explicit demonstration that imprinted genes expressed in the offspring influence maternal behaviour lends significant weight to the hypothesis that maternal care is a resource that has been manipulated by the paternal genome.

1. Genomic imprinting

Genomic imprinting is the term used to describe an epigenetic process whereby the parental alleles are marked in the germline to be differentially expressed in the offspring (Surani, 1998). In modern mammals, this remarkable process has resulted in more than 100 protein coding genes and numerous noncoding RNAs being expressed predominantly from a single parental allele. Some imprinted genes are tightly regulated and globally monoallelically expressed, some show tissue and/or temporal specificities in their imprinted expression while others are paternally or maternally expressed in different tissues through the differential use of imprinted promoters. The existence of imprinted genes was essentially predicted prior to their physical discovery through the recognition of a conflict between parent and offspring (Trivers, 1974), in particular those imposed by pregnancy (Haig, 1993). Pregnancy represents a unique and challenging dilemma for mammals as considerable and almost exclusively maternal resources are required to produce offspring but the mother must, at the same time, ensure her own survival for the production of future offspring. Resource allocation has traditionally referred to nutrient allocation both during pregnancy and postnatally (lactation) but the finding in 1998 that an imprinted gene, called *Paternally expressed gene 1* (*Peg1*) influenced maternal behaviour (Lefebvre et al., 1998) suggested that maternal care provision is another resource which can potentially be manipulated by the parental genomes.

Maternal behaviour is broadly defined as "the pattern of a mother's behaviour that appears to enhance her offspring's survival and reproductive success" (Saltzman and Maestripieri, 2011). The capacity to respond in a maternal manner is present in most, but not all, female mammals. The natural process of transitioning from a nulliparous female to a mother culminates in the initiation of enhanced maternal care towards new born offspring. These changes irreversibly alter the new mother's motivational and behavioural repertoire driving an increased interest in sensory modalities associated with young. In humans, a failure to make the appropriate transition into motherhood can have lasting effects upon maternal health in the weeks, months and years after birth. Unsurprisingly, early social experiences gained through the relationship between the new mother and her offspring also significantly affects the developmental trajectory of her offspring (Kaffman and Meaney, 2007; Cameron et al., 2017). These early life interactions are associated with fundamental changes in the brain and behaviour that persist right through into adulthood. Therefore understanding how maternal responses are primed prenatally and further stimulated from birth is important not only for maternal mental health, but also for the health of future generations.

Over the past two decades, there has been considerable progress in

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https://doi.org/10.1016/j.yfrne.2018.12.003
Received 17 September 2018; Received in revised form 15 November 2018; Accepted 12 December 2018
Available online 13 December 2018
0091-3022/

the foundational aspects of mammalian reproduction, including the processes involved in parturition, lactation, behaviour and stress response. These discoveries have predominately been made in rodents. While rodents do not recapitulate all the sophisticated aspects of humans, mice and rats have been used as a model system to study maternal behaviour because they display behaviours that are predictable and testable, making them the ideal choice of animal model. Mice, and more recently rats, are also genetically modifiable allowing functional assessment of specific genes and, with conditional models, the function of specific genes with a spatial and/or temporal focus. Rodents give birth to relative immature young (altricial) with limited mobility, incapable of fending for themselves or maintaining their own body temperature. Consequently, the new mother must exhibit maternal behaviour at parturition to ensure their immediate survival. While both virgin females and males can respond to pups, both require several days of exposure to the pups before parental behaviours emerge (Brunton and Russell, 2008).

Maternal behaviours can be categorised as direct and pup-orientated or indirect and non-pup orientated. Pup oriented behaviours are displayed as discrete acts of maternal care present from parturition. These include retrieving, grouping, crouching over pups to encourage suckling, and licking/grooming each individual pup to stimulate urination and heighten sensory stimuli developed only through nursing contact (Franks et al., 2011). Non-pup orientated behaviours encompass everything from finding and remembering food stores and water sources, identifying danger zones to building nests (Franks et al., 2011). Nests, even if already built, are re-constructed in a more elaborate and functional way for the young, to provide protection and warmth (Lisk et al., 1969). Late in pregnancy the female's aggressive behaviour increases, important for defending resources (Caughey et al., 2011). Postpartum dams show heightened aggression towards intruders stimulated by the presence of the suckling pups, and reduced anxiety thought to be important for pup focused behaviour (Bosch, 2013). Pregnant females have increased appetite throughout pregnancy to support the developing fetus and to generate surplus energy stores (fat) for lactation with further increases in feeding and drinking postpartum (Grattan, 2012). Alterations in behaviour cannot come at the cost of maternal wellbeing. Postpartum the dam must continue to maintain her wellbeing through self-grooming, enhanced feeding and drinking to ensure survival of the litter and her future reproductive health. Taken together these behaviours are necessary to maximise the level of care and fitness she can bestow upon both herself and her young.

2. Hormones and maternal behaviour

Maternal care is primed in pregnancy through the direct action of hormones on the maternal brain, both those produced by the mother and also those that originate from the fetally-derived placenta (Brunton and Russell, 2008; Grattan, 2012; Bridges, 2015). A number of neuromodulators are produced by the maternal brain and function locally in the induction and maintenance of maternal care including prolactin (Moltz et al., 1970; Bridges et al., 1985), serotonin (Angoa-Perez et al., 2014); dopamine (Scott et al., 2015; Henschen et al., 2013; Koukoulas et al., 2003), oxytocin (Fahrbach et al., 1984; Marlin et al., 2015; Parreiras et al., 2017) and vasopressin (Bosch and Neumann, 2008; Kim et al., 1997). However, initial external stimuli are required to initiate the cascade of changes that occur to the maternal brain. In mice, prolactin secretion from the pituitary is initially stimulated by mating (Shingo et al., 2003) and then secreted in surges in the afternoon and night until mid-gestation (Bridges, 2015; Soares, 2004). Prolactin has been shown to suppress the stress response in part through inhibiting the hypothalamic-pituitaryadrenal axis (Torner et al., 2001; Torner et al., 2002), to induce changes in activity of oxytocin neurons (Kokay et al., 2006), to stimulate neurogenesis (Shingo et al., 2003), to suppresses ovulation and to regulate its own secretion (Freeman et al., 2000). At mid gestation, prolactin secretion is suppressed and superseded by the placental lactogens (Prls) which are lactogenic hormones related to prolactin but produced by the fetally-derived placenta. Prls predominate for the rest of pregnancy until, the night before parturition, prolactin secretion again surges in response to progesterone withdrawal (Bridges, 2015; Soares, 2004). Prolactin stimulates maternal care (Moltz et al., 1970; Bridges et al., 1985) and prolactin action in the medial preoptic area is necessary for postpartum maternal nursing behaviour and maternal neurogenesis (Shingo et al., 2003) while low levels of prolactin are associated with increased postpartum anxiety and decreased pup retrieval, alongside a reduction in pregnancy-induced neurogenesis (Larsen and Grattan, 2012). Although the data are less clear, prolactin may also influence maternal neurogenesis in the sub granular zone of the hippocampus, important in learning and memory (Walker et al., 2012; Rolls et al., 2008). Prolactin likely functions entirely via the prolactin receptor (Prlr) as loss of function of this receptor in nulliparous females, either homozygously or heterozygously, results in a defect in foster pup–induced maternal behaviour (Lucas et al., 1998) and pregnant *Prlr*$^{-/+}$ (heterozygous) dams display a 50% reduction in forebrain neurogenesis in the subventricular zone (Shingo et al., 2003). The fact that animals with a disrupted prolactin gene still exhibit some maternal behaviour (Horseman et al., 1997), combined with the knowledge that Prl3d1 (PL-I) and Prl3b1 (PL-II) are known to bind and activate the Prlr receptor (Soares et al., 2007) suggests that these Prls contribute to the induction of maternal care, although this has only been shown indirectly through the direct infusion of placental lactogen into the medial preoptic area of nulliparous rats (Bridges and Freemark, 1995).

Lactogenic hormones are not solely responsible for initiating changes in the maternal brain. Steroid hormones (progesterone and oestradiol) produced primarily from the ovary diffuse across the blood brain barrier to act directly on the maternal brain (Bridges, 2015). Progesterone steadily increases during the pregnancy priming the female brain to respond to an acute increase in oestradiol that occurs at parturition while a fall in progesterone secretion at the end of pregnancy synchronises the onset of maternal behaviour with parturition (Bridges, 2015; Siegel and Rosenblatt, 1978; Malassine et al., 2003). Steroid hormones are produced from the ovary partly controlled by lactogenic hormones (Malassine et al., 2003) but, while the placenta expresses some stereogenic enzymes, it does not appear to play a role in *de novo* steroid synthesis, at least in rodents (Arensburg et al., 1999). Similarly, the placenta expresses components of the serotonin, dopamine, oxytocin and vasopressin synthesis pathways but expressed at such low levels (Creeth et al., 2018) that their site of action, if any, is likely to be fetal rather than maternal, as shown for serotonin (Bonnin et al., 2011). The mouse placenta manufactures other protein hormones including secretin and galanin at more functionally convincing levels (Knox et al., 2011; Wu et al., 2014). Secretin is required for hippocampal synaptic plasticity and *secretin receptor* mutant mice display abnormal social and cognitive behaviours (Yamagata et al., 2008; Nishijima et al., 2006) while galanin-expressing neurons in the maternal hypothalamus are important for maternal behaviour with activation inducing pup grooming in virgin females and ablation of these neurons resulted in postpartum females attacking rather than caring for pups (Wu et al., 2014).

The complexity of changes to the maternal brain both during pregnancy and postpartum are such that it is not surprising that a vast number of genetic modifications to the mother result in deficits in maternal behaviour. Indeed, when we surveyed the literature on genetic mutations leading to alterations in maternal behaviour, we found 64 examples reporting a deficit in maternal care and none that enhanced this behaviour (Creeth et al., 2018). The caveat is, of course, that it is much easier to spot a deficit in maternal care, particularly when there is reduced pup survival, than it is to detect improved maternal care. However, the potential for hormones manufactured by the placenta to influence the programming of the maternal brain in pregnancy suggests that placental endocrine changes driven by genetic

alterations may positively impact maternal behaviour, as now demonstrated for the first time by our lab (Creeth et al., 2018).

3. Disruptions of imprinted genes expressed in the dam that result in maternal care deficits

3.1. Paternally expressed gene 1 (Peg1)

Peg1 (aka *Mest*) was the first imprinted gene linked to maternal care in mice (Lefebvre et al., 1998). *Peg1* was initially identified as an imprinted gene through subtractive hybridisation between cDNAs from normal and parthenogenetic embryos, and found to map to the proximal region of mouse chromosome 6 (Kaneko-Ishino et al., 1995). The maternal promoter and part of exon 1 acquire DNA methylation during oocyte maturation (Lucifero et al., 2002) which is present in fetal tissues (Lefebvre et al., 1997). *Peg1* is a member of the divergent α-β hydrolase protein family where 8 β-sheets are connected by α-helices in the core of the protein (Ollis et al., 1992). During fetal development *Peg1* is predominantly expressed in mesodermal tissues including heart, lung, cartilage, skeletal muscle and tongue, and also within the remnants of Rathke's pouch, the amygdala, ventral hippocampus, main and accessory olfactory bulbs, cortex, dorsal hippocampus and striatum and the choroid plexus (Lefebvre et al., 1998; Kaneko-Ishino et al., 1995). Expression in the developing mouse midbrain overlaps with mesodiencephalic dopaminergic neurons before becoming restricted to part of the substantia nigra in adults (Mesman et al., 2016).

Loss-of-function of *Peg1* (paternal inheritance of the targeted allele) was found to result in a ~15% proportionate growth restriction of the fetus and placenta at E18.5 when studied on the 129Sv strain background (Lefebvre et al., 1998). In this study, less than 50% of *Peg1* mutants survived to adulthood and those that survived remained small with no evidence for catch-up growth. When *Peg1* mutant females (paternal inheritance of targeted allele) were mated with wild type males, they underwent a normal first pregnancy (although this was not assessed in great detail) and delivered at term. However, *Peg1* mutant dams frequently failed to remove extraembryonic membranes, eat the placenta or nurse their pups and nearly 90% of newborn died shortly after birth. In a pup retrieval task, mutant dams were found to sniff their pups indicating an intact olfactory response but did not retrieve them, and were disinterested in nest building. Neonates cross fostered to wild type foster mothers survived attributing the deficit to mutant dam rather than her offspring.

3.2. Paternally expressed gene 3 (Peg3)

Peg3 was the second imprinted gene linked to maternal care in mice (Li et al., 1999). *Peg3* was identified with *Peg1* (Kaneko-Ishino et al., 1995) and in a second screen for novel imprinted genes and maps to proximal mouse chromosome 7 (Kuroiwa et al., 1996). Like *Peg1*; *Peg3* is paternally expressed and DNA methylation spanning the promoter region is on the maternal allele, inherited from the oocyte (Lucifero et al., 2002). *Peg3* encodes a Kruppel C2H2-type zinc finger protein (Kuroiwa et al., 1996) which functions to repress gene transcription (Thiaville et al., 2013; Lee et al., 2015; Kim et al., 2013). Expression is predominantly from the paternal allele although maternal allele expression has been reported in the adult brain (Perera and Kim, 2016). During fetal development *Peg3* expression overlaps substantially with *Peg1* (Lefebvre et al., 1998; Li et al., 1999).

Loss-of-function of *Peg3* (paternal inheritance of the targeted allele) results in fetal and placental growth restriction (Li et al., 1999; Kim et al., 2013; Denizot et al., 2016). Mice are born small and remain small framed in later life but deposit excess fat despite consuming less food (Curley et al., 2005). Adult male and female mutant mice display altered behaviour with males failing to respond normally to sexually receptive females (Swaney et al., 2008) and females exhibiting deficits in classic tests of maternal behaviours in their first pregnancy (Li et al., 1999). *Peg3* mutant dams sniff their pups in the pup retrieval task but fail to return them to the nest in a timely manner, are slow to crouch over them and are poor nest builders. These original studies were made on the 129Sv stain background but despite naturally occurring differences in maternal behaviour (Champagne et al., 2007), poor quality maternal care was also apparent when this same modification was examined on a C57BL/6J strain background (Champagne et al., 2009), although not with a different modification (Denizot et al., 2016). When presented with wild type pups, parturient and nonparturient multiparous dams as well as virgin females all show impaired maternal behaviour (Champagne et al., 2009), again attributing the deficit to mutant female. These deficits may in part be explained by a reduction in oxytocin-positive neurons in the hypothalamus compared to wild-type females (Li et al., 1999; Champagne et al., 2009).

3.3. Type 3 deiodinase (Dio3)

Loss-of-function of *Dio3* (paternal inheritance of the targeted allele) has more recently been linked to a deficit in maternal care (Stohn et al., 2018). *Dio3* was identified as an imprinted gene in part due to its proximity to an imprinted gene cluster on chromosome 12 spanning *Dlk1* and *Gtl2* (Hernandez et al., 2002; Tsai et al., 2002; Takada et al., 2002). Preferential allelic expression of Dio3 from the paternal allele occurs in most but not all fetal tissues (Martinez et al., 2014) and the promoter lacks direct DNA methylation (Tsai et al., 2002). During development *Dio3* is expressed in the fetal brain, eye, palate, tongue, skeletal muscle around the digits, mesenchyme of the frontal region, upper lip, and lower jaw, liver, gut mesentery, testes and in the labyrinthine trophoblast of the placenta. *Dio3* is highly expressed in mature brain (Hernandez et al., 2002) where expression is reportedly biallelic (Martinez et al., 2014).

Dio3 encodes an enzyme which inactivates the hormones thyroxine and 3,5,3′-triiodothyronine (Hernandez, 2005). *Dio3*-deficiency leads to brain thyroid hormone excess. Female mice show normal social interest in unfamiliar female mice outside their home cage but in their home cage they are aggressive towards unfamiliar females (Stohn et al., 2018). *Dio3* mutant dams make flatter nests and show a deficiency in pup retrieval in their first but not their second pregnancy. They spend less time interacting with their pups and there is a high rate of pup mortality. *Dio3* mutant females also show a mild olfactory impairment. How much of this behaviour is really specific to pregnancy is unclear as non-pregnant females exhibit increased activity and decreased anxiety/depression-like behaviour (Stohn et al., 2018) alongside many other significant impairments (Charalambous and Hernandez, 1830).

Given the extensive and complex changes that occur in the maternal brain during pregnancy and postpartum (Bridges, 2015; Hillerer et al., 2014), it is perhaps not surprising that the genetic disruption of a number of imprinted genes in the dam result in deficits in maternal behaviour. A number of other imprinted genes are required for the normal function of the postnatal brain (Perez et al., 2016) and may also be important for maternal care. However, any finding based on global targeted deletions must be interpreted cautiously as loss-of-function of essential genes will have a catastrophic impact on many aspects of physiology and behaviour. Determining how relevant these findings are to studies on imprinting will require both dose- and brain-specific manipulations.

4. Disruptions of imprinted genes expressed in the offspring that result in maternal care deficits

4.1. Prenatal influence of imprinted genes expressed in the offspring on maternal care

Given the predicted importance of placental hormones in programming maternal care, imprinted genes expressed in the fetally derived placenta have the potential to influence maternal care before

birth through the regulation of placental hormone production (John, 2013). Until recently this has never been formally demonstrated in a physiologically relevant model. The mouse placenta is grossly composed of three histologically distinct regions: the labyrinth where nutrient exchange takes place, the junctional zone which is a major endocrine compartment and the decidua which is the maternally derived component (John and Hemberger, 2012). Seven distinct placental lineages express hormones including five trophoblast giant cell subtypes in close proximity to the maternal circulation, and the spongiotrophoblast and glycogen cell lineages located within the junctional zone (Simmons et al., 2007; Simmons et al., 2008; Gasperowicz et al., 2013; Rai and Cross, 2014). Of these, the spongiotrophoblast is the most substantial lineage in the mature mouse placenta (Coan et al., 2006). Endocrine lineages expresses *Prls* (Simmons et al., 2007; Simmons et al., 2008) and *pregnancy specific glycoproteins* (*Psgs*), a 17 member multigene gene family that modulate the maternal immune system and remodel vasculature (Moore and Dveksler, 2014). Several maternally expressed imprinted genes have been shown to regulate these lineages (John, 2013) including *Pleckstrin homology-like domain family A member 2* (*Phlda2*) (Tunster et al., 2016) and *Peg3* (Tunster et al., 2018) (Fig. 1).

4.1.1. Pleckstrin homology-like domain family A member 2 (Phlda2)

Phlda2 encodes a PH-only domain protein expressed primarily from the maternal allele in extraembryonic structures with maternal bias or biallelic expression in the embryo and adult (Frank et al., 1999). *Phlda2* is expressed in the ectoplacental cone where the progenitors of many cell types of the mature placenta reside, and in the extraembryonic membranes (the visceral endoderm of the yolk sac) (Frank et al., 1999; Dunwoodie and Beddington, 2002). From E10.5 *Phlda2* expression is restricted to type I syncitiotrophoblast and, at a lower level, type II syncitiotrophoblast cells in the labyrinth zone, and declines in expression from E14.5.

Studies from the Tycko laboratory and our research group defined a key role for *Phlda2* in the development of the placenta (Tunster et al., 2016; Frank et al., 2002; Salas et al., 2004; Tunster et al., 2010; Tunster et al., 2014). Loss-of-function (maternal inheritance of targeted allele) resulted in placental overgrowth with a substantial increase in placental glycogen but with a negative impact on late fetal growth (Tunster et al., 2016; Frank et al., 2002). Overexpression of *Phlda2* also resulted in fetal growth restriction, which we demonstrated first using at a transgenic mouse model in which *Phlda2* was overexpressed at four-fold the normal level from a bacterial artificial chromosome (BAC) transgene (Salas et al., 2004). Overexpression at both four-fold and two-fold the normal level resulted in a similar degree of growth restriction of both the fetus and the placenta (Tunster et al., 2016; Salas et al., 2004; Tunster et al., 2010; Tunster et al., 2014). Further assessment of the placental phenotype in the single copy line, effectively modelling loss of imprinting of *Phlda2* (two-fold expression), identified the specific reduction of only one placental lineage, the spongiotrophoblast (Tunster et al., 2016; Tunster et al., 2010; Tunster et al., 2014). Two-fold expression of *Phlda2* suppressed the proliferation of the spongiotrophoblast lineage from as early at E10.5 resulting in a 50% loss of this lineage by E14.5. Conversely, we were able to show that loss of *Phlda2* expression (maternal inheritance of *Phlda2* targeted allele), resulted in a substantial 200% increase in the contribution of the spongiotrophoblast lineage to the mature mouse placenta (Tunster et al., 2016). This work identified *Phlda2* as a negative rheostat controlling the ultimate contribution of the spongiotrophoblast lineage to the mature mouse placenta. This was a finding of major signficance because the spongiotrophoblast is the main endocrine lineage of the mature mouse placenta. Therefore, *Phlda2* indirectly regulates the expression of all the placental hormones expression from this lineage, which we further demonstrated by analysing the spongiotrophoblast transcriptome (Tunster et al., 2016). Specifically, higher levels of *Phlda2* effectively reduce the expression of a number of placental lactogens while lower levels of *Phlda2* result in increased expression of these same hormones (Fig. 2). Together, this led us to hypothesise that the expression of imprinted genes in the placenta might influence maternal adaptations to pregnancy by regulating the development of the placental endocrine

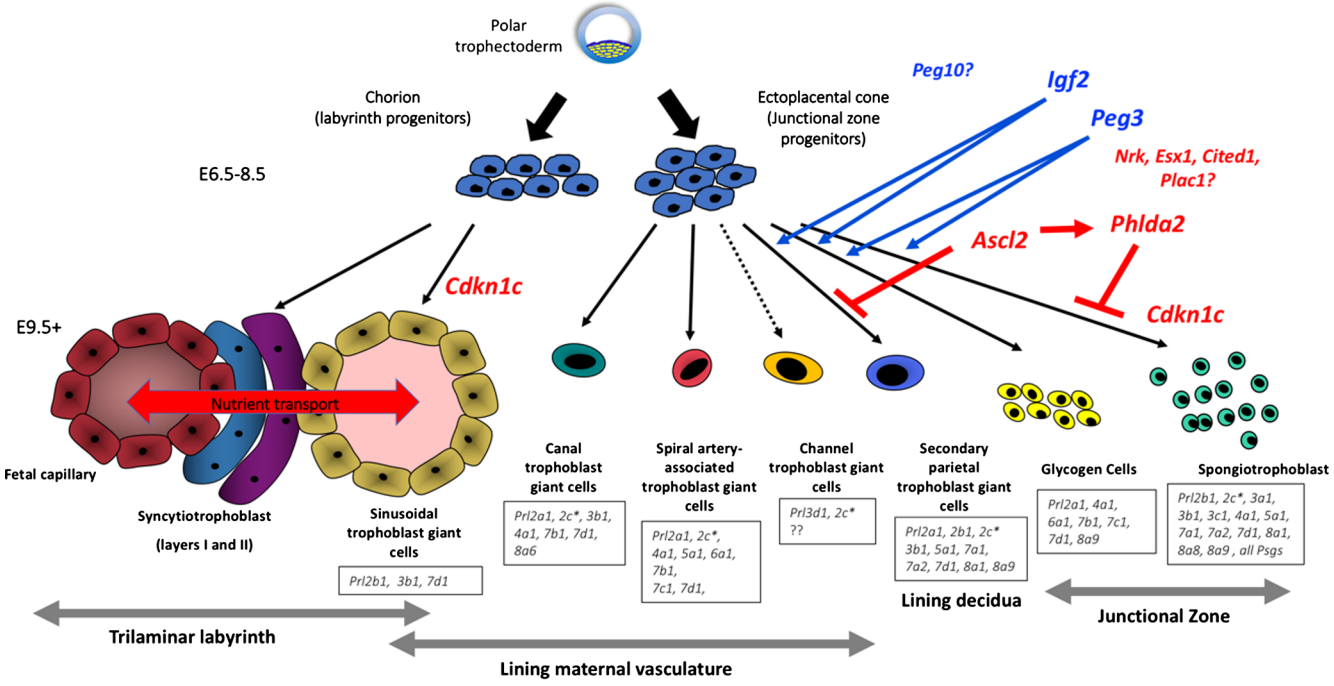

Fig. 1. Placental endocrine lineages regulated by imprinted genes. Nine distinguishable placental lineages develop from progenitors located within the ectoplacental cone and chorion. Hormones expressed from the placental endocrine lineages are shown in boxes below the cell lineages. Genomic imprinting overcomes the rapid evolution of placental hormone gene families by regulating the lineages that express hormones rather than the directly regulating the genes. Maternally expressed/paternally silenced genes (shown in red text) constrain the expansion of several endocrine lineages. Paternally expressed/maternally silenced genes (shown in blue text) boost the expansion of endocrine lineages. Figure adapted from (Tunster et al., 2018). References within text.

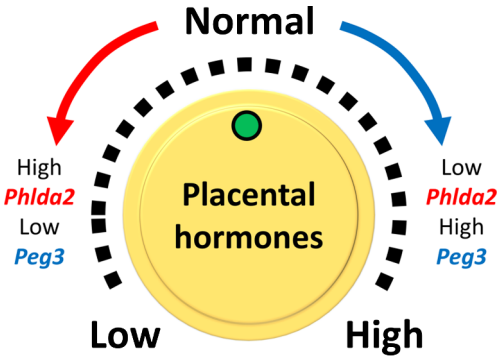

Fig. 2. Maternally and paternally expressed imprinted genes function antagonistically to regulate placental hormones. Paternalisation (blue arrow; silencing of genes on the paternal allele) boosts the expression of placental hormones while maternalisation (red arrow; silencing of genes on the maternal allele) moderates their expression. Maternally expressed/paternally silenced *Phlda2* shown in red text and paternally expressed/maternally silent *Peg3* gene shown in blue text. Consequence of high *Peg3* inferred but not shown experimentally. References within text.

lineages (John, 2013). One of the hormones negatively regulated by *Phlda2* is *Prl3b1*, a placental lactogen which binds the prolactin receptor (Soares et al., 2007). Given the known function of prolactin and the prolactin receptor in maternal care, we further hypothesised that placental *Phlda2* might also influence maternal behaviour. We tested this hypothesis by exposing wild type females to offspring expressing different doses of the Phlda2 gene (Creeth et al., 2018).

A key aspect to this study was the maintenance of the dams genetically wild type status in order to isolate the function of *Phlda2* in the offspring, achieved through recipient transfer of embryos from genetically modified parents into wild type recipients. Wild type female mice exposed to offspring with three different doses of Phlda2 – either two active alleles (loss-of-imprint), one active allele (normal imprint) or no active allele (loss of maternal allele) – showed alterations in their hypothalamic and hippocampal transcriptomes during pregnancy, regions important for maternal-care behaviour. Each group showed distinct changes that included alterations in G protein-coupled receptors (GPCR) pathways through which neuropeptides and hormones mediate their action, olfactory transduction pathways important for maternal care (Bridges, 2015; Stolzenberg and Champagne, 2016; Levy and Keller, 2009) and the gonadotropin-releasing hormone signalling pathway, implicated in maternal care and known to respond to prolactin (Brooks et al., 2012; Grattan et al., 2007). In contrast to our prediction that increased placental hormone would translate to "better mothers", in the pup retrieval task dams exposed to higher levels of placental hormones in pregnancy (loss of *Phlda2* expression) took significantly longer to retrieve their first pups than either fully wild type dams or those exposed to the lower dose of hormones (loss of *Phlda2* imprint). Similarly, in the nest building task the dams we predicted would be better at this task performed very poorly, with only one dam building a nest and putting her pups inside the nest. In stark contrast, the dams we predicted would be "worse mothers" (exposed to less hormones) actually performed better even than the fully wild type controls in nest building. However, in an undisturbed situation, all dams were able to effectively make nests and gather their pups within the nest. Pups gained weight appropriately arguing against a deficit in lactation, and pup ultrasonic vocalisations (USVs) were normal when pups were separated from their mothers. A further exploration of dams' behaviour in the disturbed situation (nest building task) revealed that dams exposed to higher levels of placental hormones in pregnancy were prioritising caring for their pups (licking and grooming) and themselves (self-grooming, feeding) over the nest building. As a final test, we asked whether dams exposed *in utero* to higher levels of placental hormones maintained their enhanced nurturing behaviour when presented with wild type pups from birth. These exposed dams similarly displayed enhanced nurturing towards the wild type fostered pups despite a considerable disruption imposed by the fostering process, supporting our original hypothesis that the priming of maternal care occurred prenatally. Overall the changes in maternal behaviour we observed were subtle and did not have a negative impact on either maternal or pup welfare, at least in the short term evidenced by appropriate pup weight gain. However, we do not know what the longer term consequence will be either for the dams in their second pregnancy, not for the offspring exposed to less optimal maternal care. Nor have we explored the neural changes or identified the specific placental hormone (s) mediating the relationship between the placenta and the maternal brain. Given the requirement for two copies of a functional *prolactin receptor* gene in maternal care (Shingo et al., 2003; Lucas et al., 1998), and the knowledge that Prl3b1 binds this receptor (Soares et al., 2007), this seems a promising avenue to explore. Irrespective of the gaps still remaining in our knowledge, this work firstly demonstrates the importance of optimal placental function in maternal care, at least in rodents, and highlights a new role for genomic imprinting in influencing maternal care giving before the offspring are born (Fig. 3).

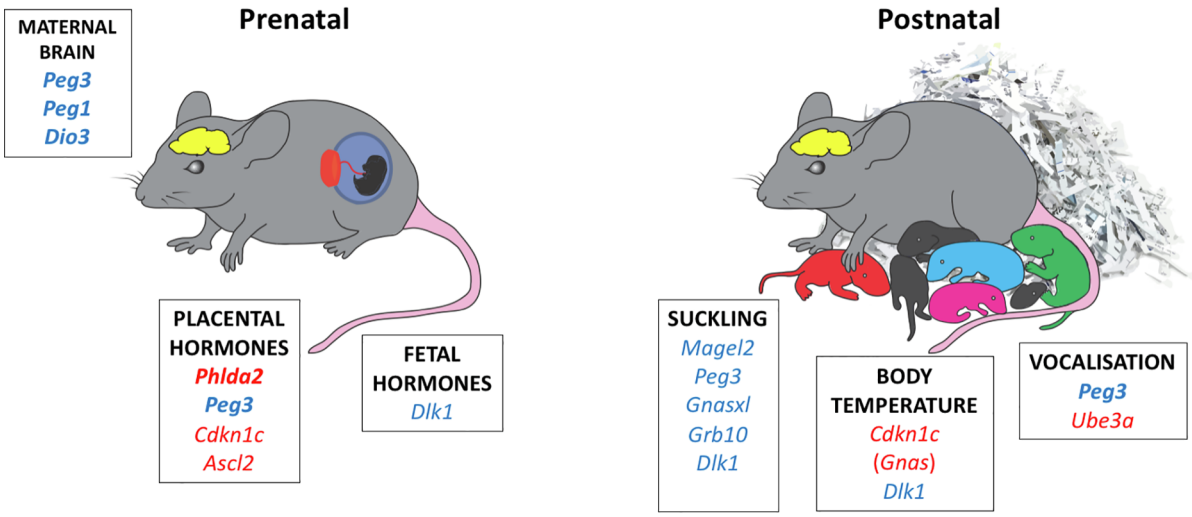

Fig. 3. Summary of imprinted genes known (bold italics) or with potential (non-bold italics) to influence maternal behaviour. Maternally expressed/paternally silenced genes shown in red text and paternally expressed/maternally silenced genes shown in blue text. References within text.

4.1.2. Peg3

As has already been discussed, loss-of-function of *Peg3* in adult female mice negatively affects their maternal behaviour. *Peg3* is expressed in both the developing fetus (Hiby et al., 2001) and in the placenta in spongiotrophoblast, glycogen cell and all but the spiral artery trophoblast giant cell lineages (Tunster et al., 2018). Microarray studies on placental gene expression in response to loss of *Peg3* suggested an impact on placental hormones (Kim et al., 2013; Broad and Keverne, 2011) and we reported a substantial loss of both the spongiotrophoblast and the glycogen cell lineage in *Peg3* deficient placenta (Tunster et al., 2018). Together with our data on *Phlda2*, this led us to speculate that placental *Peg3* may also function prenatally to influence maternal behaviour, potentially in an antagonistic manner to *Phlda2* (Fig. 2). To test this hypothesis, we generated females carrying litters of all *Peg3* mutant pups, this time by natural mating of wild type dams with males homozygous for the *Peg3* targeted allele (McNamara et al., 2018). We observed subtle changes in maternal novelty reactivity during pregnancy but no significant alterations in gene expression in the prenatal hypothalamus or hippocampus, at least at E16.5. After birth, dams exposed to *Peg3* mutant pups were slower to retrieve but showed no differences in nest building or the care of their pups during the nest building task. During the elevated zero maze task the exposed dams showed evidence of increased anxiety spending less time on the open arm. When we measured communications from pups when separated from the dam, we found that the *Peg3* mutant pups displayed fewer calls. These ultrasonic vocalisations (USVs) normally increase in intensity and frequency when pups are separated from their mothers – hence the alternative and more forlorn term – “whistles of loneliness” (Zippelius and Schleidt, 1956). They are essential for the manisfestation of maternal behaviours such as nest building, pup retrieval and nursing (Wohr and Schwarting, 2013; Scattoni et al., 2009; D'Amato et al., 2005; Okabe et al., 2013). Together, these data suggest that *Peg3* predominantly influences maternal care in the post-natal period through influencing direct communication to the mother. A major caveat to this conclusion is that we did not undertake a cross fostering experiment to isolate the pre- and post-natal influence of *Peg3*. A further caveat is our finding of the sexually dimorphic placental endocrine phenotype in *Peg3* mutant mice (Tunster et al., 2018). While loss of function of *Peg3* resulted in a substantial loss of both the spongiotrophoblast and the glycogen cell lineage in male placenta, female placenta showing an attenuated phenotype. Furthermore, when we examined the expression of placental hormones, female placenta showed very few alterations and some placental hormones were actually expressed at higher levels, despite the loss of spongiotrophoblast cells expressing these hormones. This finding is explained through a second sexually dimorphic function of *Peg3* as a transcriptional repressor of some placental hormone genes (Kim et al., 2013). Effectively, loss of *Peg3* results in fewer endocrine cells expressing higher levels of a subset of hormones both positively and negatively regulating the production of placental hormones with different consequences for male and female placenta. This new observation adds a further layer of complexity to our interpretation of the maternal behavioural changes in response to loss of offspring *Peg3*. Since mouse litters are composed of both male and female foetuses, the presence of female placenta may “rescue” deficits in the mutant male placenta.

4.1.3. Other genes with the potential to influence maternal behaviour prenatally

In addition to *Phlda2* and *Peg3* there are several other imprinted genes that regulate the placental endocrine lineage in mice including *cyclin dependent kinase inhibitor 1C* (*Cdkn1c)* (Tunster et al., 2011); *Achaete-scute complex homolog 2 (Ascl2)* (Tunster et al., 2016); *Insulin-like growth factor 2 (Igf2)* (Esquiliano et al., 2009); *Paternally expressed gene 10* (*Peg10)* (Ono et al., 2006) and four genes expressed from the X-chromosome which is paternally silenced in the mouse placenta (John, 2013). In addition to these imprinted genes that regulate the contribution of key endocrine lineages to the mature mouse placenta, some imprinted genes encode hormones which can directly act on the maternal circulation such as *Delta Like Non-Canonical Notch Ligand 1* (*Dlk1*) (Cleaton et al., 2016). All of these genes have the potential to impact maternal behaviour either directly by acting on the brain or potentially indirectly by impacting other aspects of pregnancy (Fig. 3).

4.2. Postnatal influence of imprinted genes expressed in the offspring on maternal care

Postpartum the presence of pups is clearly a requisit for the manifestation of maternal behaviour and mutations impacting pup characteristics have the potential to impact maternal behaviour. Stimulation of dams by the act of suckling (but not lactation *per se*) initiates and maintains maternal aggression postpartum by stimulating oxytocin release from the maternal hypothalamus (Svare et al., 1980; Svare and Gandelman, 1976). Loss of function *MAGE Family Member L2* (*Magel2*) (Schaller et al., 2010); *Peg3* (Curley et al., 2004); *G protein alpha-subunit xl (GnasXL)* (Plagge et al., 2004) and *Growth factor receptor-bound substrate 10* (*Grb10*) (Cowley et al., 2014) have all been reported to impact the ability of the pups to suckle with the potential of impacting maternal aggression. Communication between pups and dams is an essential component of this developing relationship. As described in detail earlier, loss-of-function of *Peg3* results in decreased pup vocalisations, alongside decreased pup retrieval by wild type mothers, and increased anxiety (McNamara et al., 2018). Increased expression of the maternally expressed *Ubiquitin-protein ligase E3A* (Ube3a) also impairs vocal communications (Smith et al., 2011) while loss of function of genes spanning Ube3a (Jiang et al., 2010) increases pup USVs potentially functioning antagonistically to *Peg3* (Fig. 3). Even the pup’s ability to maintain their own body temperature could influence maternal behaviour requiring increased maternal 'crouching', elevating demand on maternal resources. Several imprinted genes have been reported to play a role regulating brown adipogenesis impacting thermogenesis (body warmth) including *Dlk1* (Charalambous et al., 2012); *Cdkn1c* (Van De Pette et al., 2016) and *Gnas* (Paulo et al., 2018) (Fig. 3). However, with the exception of *Peg3* (McNamara et al., 2018), nothing is known about the behaviour of wildtype mothers in the context of these models.

Overall, these experiments highlight the bidirectional relationship between the mother and her offspring. *Phlda2*, which has been silenced by the paternal genome, functions normally to repress the production of placental hormones whereas *Peg3*, silenced by the maternal germline, functions to promote the production of placental hormones, and is also required for pups to effectively communicate to their mothers. During the evolution of mammals, paternalisation of *Phlda2* may have boosted maternal care via the increased production of placental hormones whereas maternalisation of *Peg3* may have decreased maternal care by decreasing the production of placental hormone and decreasing the vocal demands from the pup (Fig. 3). *Phlda2* is not imprinted in marsupials (Suzuki et al., 2011) but it is not known when, during the evolution of mammals, *Peg3* acquired an imprinted status (Suzuki et al., 2011). Nonetheless, this pre- and post-natal sequence of events suggests a continual and subtle rebalancing of maternal care allocation in mammals through the action of imprinted genes. Moreover, based on their function in regulating placental endocrine lineages (John, 2013), we predict that a number of other imprinted genes will similarly influence maternal care giving.

5. Relevance to humans

Studies on the function of imprinted genes in instructing maternal behaviour have been performed solely in mice. However, there is indirect data to suggest that these genes may have some similar functions in other mammals including humans. Higher placental expression of *PHLDA2* has been reported in association with low birth weight in a

number of studies (Jensen et al., 2014). Traditionally, infants are classified as low birth weight if born weighing less than 2.5 kg at any gestational age (United Nations Children's Fund and World Health Organisation 2004). Low birth weight can be a consequence of a premature birth or fetal growth restriction where the baby has not reached its genetic growth potential, or both factors. Mice with two-fold expression of *Phlda2*, similar to the level reported in affected human pregnancies, display a slowdown in fetal growth late in gestation and are born low birth weight supporting a causal role for elevated *PHLDA2* in driving fetal growth restriction and consequent low birth weight in human pregnancies. The inverse correlation between placental *PHLDA2* expression and maternal serum levels of human placental lactogen (Janssen et al., 2016) further suggests that *PHLDA2* negatively regulates the production of placental hormones in humans as well as mice. Although there are substantial differences in the placental structure between man and mouse, and also in the type and function of placental hormones (Carter, 2012), we suggest the possibility that *PHLDA2* may have a role in the induction of maternal care in human pregnancies. Little is known about the quality of care mothers provide to their low birth weight newborn, and any observations are likely confounded by multiple factors surrounding the birth. However, while maternal care and maternal depression are two very different facets of pregnancy, depression could be a manifestation of miss programmed maternal behaviour in pregnancy. Both preterm birth and low birth weight are more common in pregnancies where mothers report depressive symptoms (Grote et al., 2010) and mothers of preterm and low birth infants are more likely to suffer postpartum depression (Vigod et al., 2010). In our study of placental gene expression in relation to prenatal clinically diagnosed depression, we did not find any association with *PHLDA2* expression levels in the placenta (Janssen et al., 2016). However, this was a small number of women and we cannot formally exclude a role for placental *PHLDA2* in maternal mood disorders or postnatal care provision. In this same study we did find markedly reduced expression of *PEG3* in both the placenta from pregnancies where women were clinically diagnosed with depression and also those pregnancies where women self-reported depressive symptoms in pregnancy. This association was only apparent in the placenta from boys exposed to depression and not girls. Given our finding of a sexually dimorphic response of the mouse placenta to loss of Peg3 function (Tunster et al., 2018), this lends support to a causal role for reduced *Peg3* contributing to depression in human pregnancies. While we did not observe indicators of depressive symptoms in our *Peg3* mouse model, we did not use extensive tests for depression due to the pregnant status of the dams but dams were more anxious postpartum. Depression and anxiety are comorbid in human pregnancies and further work will be is essential to distinguish between the prenatal impact of placental dysfunction and the postnatal impact of reduced communication in this model.

6. Summary

In summary, imprinted genes function in many aspects of development and behaviour but the often the catastrophic impact of loss of function can preclude a meaningful interpretation of the function of the imprint. Demonstrating that imprinted genes expressed in the offspring can influence maternal behaviour (Creeth et al., 2018; McNamara et al., 2018), and more critically, boost the quality of maternal care, lends much greater weight to the idea that maternal care provision is a resource which is manipulated by the parental genomes.

Acknowledgements

HDJC was supported by BBSRC grant BB/P002307 and GIM was supported by MRC grant MR/M013960.

Competing interests statement

Since September 2017, GMN has been employed by Frontiers Media SA. GMN declared her affiliation with Frontiers. RMJ is chief Specialty editor of Frontiers in Cell and Development Biology. The other authors declare that the review was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yfrne.2018.12.003.

References

Angoa-Perez, M., Kane, M.J., Sykes, C.E., Perrine, S.A., Church, M.W., Kuhn, D.M., 2014. Brain serotonin determines maternal behavior and offspring survival. Genes Brain Behav. 13, 579–591.
Arensburg, J., Payne, A.H., Orly, J., 1999. Expression of steroidogenic genes in maternal and extraembryonic cells during early pregnancy in mice. Endocrinology 140, 5220–5232.
Bonnin, A., Goeden, N., Chen, K., Wilson, M.L., King, J., Shih, J.C., Blakely, R.D., Deneris, E.S., Levitt, P., 2011. A transient placental source of serotonin for the fetal forebrain. Nature 472, 347–350.
Bosch, O.J., 2013. Maternal aggression in rodents: brain oxytocin and vasopressin mediate pup defence. Philos. Trans. R. Soc. Lond. B Biol. Sci. 368, 20130085.
Bosch, O.J., Neumann, I.D., 2008. Brain vasopressin is an important regulator of maternal behavior independent of dams' trait anxiety. Proc. Natl. Acad. Sci. U.S.A. 105, 17139–17144.
Bridges, R.S., 2015. Neuroendocrine regulation of maternal behavior. Front. Neuroendocrinol. 36, 178–196.
Bridges, R.S., Freemark, M.S., 1995. Human placental lactogen infusions into the medial preoptic area stimulate maternal behavior in steroid-primed, nulliparous female rats. Horm. Behav. 29, 216–226.
Bridges, R.S., DiBiase, R., Loundes, D.D., Doherty, P.C., 1985. Prolactin stimulation of maternal behavior in female rats. Science 227, 782–784.
Broad, K.D., Keverne, E.B., 2011. Placental protection of the fetal brain during short-term food deprivation. Proc. Natl. Acad. Sci. U.S.A. 108, 15237–15241.
Brooks, L.R., Le, C.D., Chung, W.C., Tsai, P.S., 2012. Maternal behavior in transgenic mice with reduced fibroblast growth factor receptor function in gonadotropin-releasing hormone neurons. Behav. Brain Funct. 8, 47.
Brunton, P.J., Russell, J.A., 2008. The expectant brain: adapting for motherhood. Nat. Rev. Neurosci. 9, 11–25.
Cameron, J.L., Eagleson, K.L., Fox, N.A., Hensch, T.K., Levitt, P., 2017. Social origins of developmental risk for mental and physical illness. J. Neurosci. 37, 10783–10791.
Carter, A.M., 2012. Evolution of placental function in mammals: the molecular basis of gas and nutrient transfer, hormone secretion, and immune responses. Physiol. Rev. 92, 1543–1576.
Caughey, S.D., Klampfl, S.M., Bishop, V.R., Pfoertsch, J., Neumann, I.D., Bosch, O.J., Meddle, S.L., 2011. Changes in the intensity of maternal aggression and central oxytocin and vasopressin V1a receptors across the peripartum period in the rat. J. Neuroendocrinol. 23, 1113–1124.
Champagne, F.A., Curley, J.P., Keverne, E.B., Bateson, P.P., 2007. Natural variations in postpartum maternal care in inbred and outbred mice. Physiol. Behav. 91, 325–334.
Champagne, F.A., Curley, J.P., Swaney, W.T., Hasen, N.S., Keverne, E.B., 2009. Paternal influence on female behavior: the role of Peg3 in exploration, olfaction, and neuroendocrine regulation of maternal behavior of female mice. Behav. Neurosci. 123, 469–480.
Charalambous, M., Hernandez, A., 1830. Genomic imprinting of the type 3 thyroid hormone deiodinase gene: regulation and developmental implications. Biochim. Biophys. Acta 2013, 3946–3955.
Charalambous, M., Ferron, S.R., da Rocha, S.T., Murray, A.J., Rowland, T., Ito, M., Schuster-Gossler, K., Hernandez, A., Ferguson-Smith, A.C., 2012. Imprinted gene dosage is critical for the transition to independent life. CellMetab 15, 209–221.
Cleaton, M.A., Dent, C.L., Howard, M., Corish, J.A., Gutteridge, I., Sovio, U., Gaccioli, F., Takahashi, N., Bauer, S.R., Charnock-Jones, D.S., Powell, T.L., Smith, G.C., Ferguson-Smith, A.C., Charalambous, M., 2016. Fetus-derived DLK1 is required for maternal metabolic adaptations to pregnancy and is associated with fetal growth restriction. Nat. Genet.
Coan, P.M., Conroy, N., Burton, G.J., Ferguson-Smith, A.C., 2006. Origin and characteristics of glycogen cells in the developing murine placenta. Dev. Dyn. 235, 3280–3294.
Cowley, M., Garfield, A.S., Madon-Simon, M., Charalambous, M., Clarkson, R.W., Smalley, M.J., Kendrick, H., Isles, A.R., Parry, A.J., Carney, S., Oakey, R.J., Heisler, L.K., Moorwood, K., Wolf, J.B., Ward, A., 2014. Developmental programming mediated by complementary roles of imprinted Grb10 in mother and pup. PLoS Biol. 12, e1001799.
Creeth, H.D.J., McNamara, G.I., Tunster, S.J., Boque-Sastre, R., Allen, B., Sumption, L., Eddy, J.B., Isles, A.R., John, R.M., 2018. Maternal care boosted by paternal imprinting in mammals. PLoS Biol. 16 (7).
Curley, J.P., Barton, S., Surani, A., Keverne, E.B., 2004. Coadaptation in mother and

infant regulated by a paternally expressed imprinted gene. Proc. Biol. Sci. 271, 1303–1309.
Curley, J.P., Pinnock, S.B., Dickson, S.L., Thresher, R., Miyoshi, N., Surani, M.A., Keverne, E.B., 2005. Increased body fat in mice with a targeted mutation of the paternally expressed imprinted gene Peg3. Faseb. J. 19, 1302–1304.
D'Amato, F.R., Scalera, E., Sarli, C., Moles, A., 2005. Pups call, mothers rush: does maternal responsiveness affect the amount of ultrasonic vocalizations in mouse pups? Behav. Genet. 35, 103–112.
Denizot, A.L., Besson, V., Correra, R.M., Mazzola, A., Lopes, I., Courbard, J.R., Marazzi, G., Sassoon, D.A., 2016. A novel mutant allele of Pw1/Peg3 does not affect maternal behavior or nursing behavior. PLoS Genet. 12, e1006053.
Dunwoodie, S.L., Beddington, R.S., 2002. The expression of the imprinted gene Ipl is restricted to extra-embryonic tissues and embryonic lateral mesoderm during early mouse development. Int. J. Dev. Biol. 46, 459–466.
Esquiliano, D.R., Guo, W., Liang, L., Dikkes, P., Lopez, M.F., 2009. Placental glycogen stores are increased in mice with H19 null mutations but not in those with insulin or IGF type 1 receptor mutations. Placenta 30, 693–699.
Fahrbach, S.E., Morrell, J.I., Pfaff, D.W., 1984. Oxytocin induction of short-latency maternal behavior in nulliparous, estrogen-primed female rats. Horm. Behav. 18, 267–286.
Frank, D., Mendelsohn, C.L., Ciccone, E., Svensson, K., Ohlsson, R., Tycko, B., 1999. A novel pleckstrin homology-related gene family defined by Ipl/Tssc3, TDAG51, and Tih1: tissue-specific expression, chromosomal location, and parental imprinting. Mamm. Genome. 10, 1150–1159.
Frank, D., Fortino, W., Clark, L., Musalo, R., Wang, W., Saxena, A., Li, C.M., Reik, W., Ludwig, T., Tycko, B., 2002. Placental overgrowth in mice lacking the imprinted gene Ipl. Proc. Natl. Acad. Sci. U.S.A. 99, 7490–7495.
Franks, B., Curley, J.P., Champagne, F.A., 2011. Measuring variations in maternal behavior: relevance for studies of mood and anxiety. In: Gould, T.D. (Ed.), Mood and Anxiety Related Phenotypes in Mice. Humana Press, Humana Press.
Freeman, M.E., Kanyicska, B., Lerant, A., Nagy, G., 2000. Prolactin: structure, function, and regulation of secretion. Physiol. Rev. 80, 1523–1631.
Gasperowicz, M., Surmann-Schmitt, C., Hamada, Y., Otto, F., Cross, J.C., 2013. The transcriptional co-repressor TLE3 regulates development of trophoblast giant cells lining maternal blood spaces in the mouse placenta. Dev. Biol. 382, 1–14.
Grattan, D., 2012. A mother's brain knows. J. Neuroendocrinol. 23, 1188–1189.
Grattan, D.R., Jasoni, C.L., Liu, X., Anderson, G.M., Herbison, A.E., 2007. Prolactin regulation of gonadotropin-releasing hormone neurons to suppress luteinizing hormone secretion in mice. Endocrinology 148, 4344–4351.
Grote, N.K., Bridge, J.A., Gavin, A.R., Melville, J.L., Iyengar, S., Katon, W.J., 2010. A meta-analysis of depression during pregnancy and the risk of preterm birth, low birth weight, and intrauterine growth restriction. Arch. Gen. Psychiat. 67, 1012–1024.
Haig, D., 1993. Genetic conflicts in human pregnancy. Q. Rev. Biol. 68, 495–532.
Henschen, C.W., Palmiter, R.D., Darvas, M., 2013. Restoration of dopamine signaling to the dorsal striatum is sufficient for aspects of active maternal behavior in female mice. Endocrinology 154, 4316–4327.
Hernandez, A., 2005. Structure and function of the type 3 deiodinase gene. Thyroid 15, 865–874.
Hernandez, A., Fiering, S., Martinez, E., Galton, V.A., St Germain, D., 2002. The gene locus encoding iodothyronine deiodinase type 3 (Dio3) is imprinted in the fetus and expresses antisense transcripts. Endocrinology 143, 4483–4486.
Hiby, S.E., Lough, M., Keverne, E.B., Surani, M.A., Loke, Y.W., King, A., 2001. Paternal monoallelic expression of PEG3 in the human placenta. Hum. Mol. Genet. 10, 1093–1100.
Hillerer, K.M., Jacobs, V.R., Fischer, T., Aigner, L., 2014. The maternal brain: an organ with peripartal plasticity. Neural. Plast. 2014, 574159.
Horseman, N.D., Zhao, W., Montecino-Rodriguez, E., Tanaka, M., Nakashima, K., Engle, S.J., Smith, F., Markoff, E., Dorshkind, K., 1997. Defective mammopoiesis, but normal hematopoiesis, in mice with a targeted disruption of the prolactin gene. EMBO J. 16, 6926–6935.
Janssen, A.B., Tunster, S.J., Heazell, A.E., John, R.M., 2016. Placental PHLDA2 expression is increased in cases of fetal growth restriction following reduced fetal movements. BMC Med. Genet. 17, 17.
Janssen, A.B., Capron, L.E., O'Donnell, K., Tunster, S.J., Ramchandani, P.G., Heazell, A.E., Glover, V., John, R.M., 2016. Maternal prenatal depression is associated with decreased placental expression of the imprinted gene PEG3. Psychol. Med. 46, 2999–3011.
Jensen, A.B., Tunster, S.J., John, R.M., 2014. The significance of elevated placental PHLDA2 in human growth restricted pregnancies. Placenta 35, 528–532.
Jiang, Y.H., Pan, Y., Zhu, L., Landa, L., Yoo, J., Spencer, C., Lorenzo, I., Brilliant, M., Noebels, J., Beaudet, A.L., 2010. Altered ultrasonic vocalization and impaired learning and memory in Angelman syndrome mouse model with a large maternal deletion from Ube3a to Gabrb3. PloS One 5, e12278.
John, R.M., 2013. Epigenetic regulation of placental endocrine lineages and complications of pregnancy. Biochem. Soc. Trans. 41, 701–709.
John, R., Hemberger, M., 2012. A placenta for life. Reprod. Biomed. Online 25, 5–11.
Kaffman, A., Meaney, M.J., 2007. Neurodevelopmental sequelae of postnatal maternal care in rodents: clinical and research implications of molecular insights. J. Child Psychol. Psychiatry 48, 224–244.
Kaneko-Ishino, T., Kuroiwa, Y., Miyoshi, N., Kohda, T., Suzuki, R., Yokoyama, M., Viville, S., Barton, S.C., Ishino, F., Surani, M.A., 1995. *Peg1/Mest* imprinted gene on chromosome 6 identified by cDNA subtraction hybridization. Nat. Genet. 11, 52–59.
Kim, J., Frey, W.D., He, H., Kim, H., Ekram, M.B., Bakshi, A., Faisal, M., Perera, B.P., Ye, A., Teruyama, R., 2013. Peg3 mutational effects on reproduction and placenta-specific gene families. PloS One 8, e83359.
Kim, M.O., Kim, J.H., Choi, W.S., Lee, B.H., Cho, G.J., Roh, S.M., Lee, B.J., Kang, S.G., Kim, C.H., Baik, S.H., 1997. Colocalization of dopamine D1 and D2 receptor mRNAs in rat placenta. Mol. Cells 7, 710–714.
Knox, K., Leuenberger, D., Penn, A.A., Baker, J.C., 2011. Global hormone profiling of murine placenta reveals Secretin expression. Placenta 32, 811–816.
Kokay, I.C., Bull, P.M., Davis, R.L., Ludwig, M., Grattan, D.R., 2006. Expression of the long form of the prolactin receptor in magnocellular oxytocin neurons is associated with specific prolactin regulation of oxytocin neurons. Am. J. Physiol. Regul. Integr. Comp. Physiol. 290, R1216–R1225.
Koukoulas, I., Risvanis, J., Douglas-Denton, R., Burrell, L.M., Moritz, K.M., Wintour, E.M., 2003. Vasopressin receptor expression in the placenta. Biol. Reprod. 69, 679–686.
Kuroiwa, Y., Kaneko-Ishino, T., Kagitani, F., Kohda, T., Li, L.L., Tada, M., Suzuki, R., Yokoyama, M., Shiroishi, T., Wakana, S., Barton, S.C., 1996. *Peg3* imprinted gene on proximal chromosome 7 encodes for a zinc finger protein. Nat. Genet. 12, 186–190.
Larsen, C.M., Grattan, D.R., 2012. Prolactin, neurogenesis, and maternal behaviors. Brain Behav. Immun. 26, 201–209.
Lee, S., Ye, A., Kim, J., 2015. DNA-Binding Motif of the Imprinted Transcription Factor PEG3. PloS One 10, e0145531.
Lefebvre, L., Viville, S., Barton, S.C., Ishino, F., Surani, M.A., 1997. Genomic structure and parent-of-origin-specific methylation of Peg1. Hum. Mol. Genet. 6, 1907–1915.
Lefebvre, L., Viville, S., Barton, S.C., Ishino, F., Keverne, E.B., Surani, M.A., 1998. Abnormal maternal behaviour and growth retardation associated with loss of the imprinted gene Mest [see comments]. Nat. Genet. 20, 163–169.
Levy, F., Keller, M., 2009. Olfactory mediation of maternal behavior in selected mammalian species. Behav. Brain Res. 200, 336–345.
Li, L., Keverne, E.B., Aparicio, S.A., Ishino, F., Barton, S.C., Surani, M.A., 1999. Regulation of maternal behavior and offspring growth by paternally expressed Peg3. Science 284, 330–333.
Lisk, R.D., Pretlow, R.A., Friedman, S.M., 1969. Hormonal stimulation necessary for elicitation of maternal nest-building in the mouse (Mus musculus). Anim. Behav. 17, 730–737.
Lucas, B.K., Ormandy, C.J., Binart, N., Bridges, R.S., Kelly, P.A., 1998. Null mutation of the prolactin receptor gene produces a defect in maternal behavior. Endocrinology 139, 4102–4107.
Lucifero, D., Mertineit, C., Clarke, H.J., Bestor, T.H., Trasler, J.M., 2002. Methylation dynamics of imprinted genes in mouse germ cells. Genomics 79, 530–538.
Malassine, A., Frendo, J.L., Evain-Brion, D., 2003. A comparison of placental development and endocrine functions between the human and mouse model. Hum. Reprod. Update 9, 531–539.
Marlin, B.J., Mitre, M., Damour, J.A., Chao, M.V., Froemke, R.C., 2015. Oxytocin enables maternal behaviour by balancing cortical inhibition. Nature 520, 499–504.
Martinez, M.E., Charalambous, M., Saferali, A., Fiering, S., Naumova, A.K., St Germain, D., Ferguson-Smith, A.C., Hernandez, A., 2014. Genomic imprinting variations in the mouse type 3 deiodinase gene between tissues and brain regions. Mol. Endocrinol. 28, 1875–1886.
McNamara, G.I., Creeth, H.D.J., Harrison, D.J., Tansey, K.E., Andrews, R.M., Isles, A.R., John, R.M., 2018. Loss of offspring Peg3 reduces neonatal ultrasonic vocalizations and increases maternal anxiety in wild-type mothers. Hum. Mol. Genet. 27, 440–450.
Mesman, S., van Hooft, J.A., Smidt, M.P., 2016. Mest/Peg1 Is Essential for the Development and Maintenance of a SNc Neuronal Subset. Front. Mol. Neurosci. 9, 166.
Moltz, H., Lubin, M., Leon, M., Numan, M., 1970. Hormonal induction of maternal behavior in the ovariectomized nulliparous rat. Physiol. Behav. 5, 1373–1377.
Moore, T., Dveksler, G.S., 2014. Pregnancy-specific glycoproteins: complex gene families regulating maternal-fetal interactions. Int. J. Dev. Biol. 58, 273–280.
Nishijima, I., Yamagata, T., Spencer, C.M., Weeber, E.J., Alekseyenko, O., Sweatt, J.D., Momoi, M.Y., Ito, M., Armstrong, D.L., Nelson, D.L., Paylor, R., Bradley, A., 2006. Secretin receptor-deficient mice exhibit impaired synaptic plasticity and social behavior. Hum. Mol. Genet. 15, 3241–3250.
Okabe, S., Nagasawa, M., Kihara, T., Kato, M., Harada, T., Koshida, N., Mogi, K., Kikusui, T., 2013. Pup odor and ultrasonic vocalizations synergistically stimulate maternal attention in mice. Behav. Neurosci. 127, 432–438.
Ollis, D.L., Cheah, E., Cygler, M., Dijkstra, B., Frolow, F., Franken, S.M., Harel, M., Remington, S.J., Silman, I., Schrag, J., et al., 1992. The alpha/beta hydrolase fold. Protein Eng. 5, 197–211.
Ono, R., Nakamura, K., Inoue, K., Naruse, M., Usami, T., Wakisaka-Saito, N., Hino, T., Suzuki-Migishima, R., Ogonuki, N., Miki, H., Kohda, T., Ogura, A., Yokoyama, M., Kaneko-Ishino, T., Ishino, F., 2006. Deletion of Peg10, an imprinted gene acquired from a retrotransposon, causes early embryonic lethality. Nat. Genet. 38, 101–106.
Parreiras, E.S.L.T., Vargas-Pinilla, P., Duarte, D.A., Longo, D., Espinoza Pardo, G.V., Dulor Finkler, A., Paixao-Cortes, V.R., Pare, P., Rovaris, D.L., Oliveira, E.B., Caceres, R.A., Goncalves, G.L., Bouvier, M., Salzano, F.M., Lucion, A.B., Costa-Neto, C.M., Bortolini, M.C., 2017. Functional New World monkey oxytocin forms elicit an altered signaling profile and promotes parental care in rats. Proc. Natl. Acad. Sci. U.S.A. 114, 9044–9049.
Paulo, E., Wu, D., Wang, Y., Zhang, Y., Wu, Y., Swaney, D.L., Soucheray, M., Jimenez-Morales, D., Chawla, A., Krogan, N.J., Wang, B., 2018. Sympathetic inputs regulate adaptive thermogenesis in brown adipose tissue through cAMP-Salt inducible kinase axis. Sci. Rep. 8, 11001.
Perera, B.P., Kim, J., 2016. Alternative promoters of Peg3 with maternal specificity. Sci. Rep. 6, 24438.
Perez, J.D., Rubinstein, N.D., Dulac, C., 2016. New perspectives on genomic imprinting, an essential and multifaceted mode of epigenetic control in the developing and adult brain. Annu. Rev. Neurosci. 39, 347–384.
Plagge, A., Gordon, E., Dean, W., Boiani, R., Cinti, S., Peters, J., Kelsey, G., 2004. The imprinted signaling protein XL alpha s is required for postnatal adaptation to feeding. Nat. Genet. 36, 818–826.

Rai, A., Cross, J.C., 2014. Development of the hemochorial maternal vascular spaces in the placenta through endothelial and vasculogenic mimicry. Dev. Biol. 387, 131–141.
Rolls, A., Schori, H., London, A., Schwartz, M., 2008. Decrease in hippocampal neurogenesis during pregnancy: a link to immunity. Mol. Psychiatry 13, 468–469.
Salas, M., John, R., Saxena, A., Barton, S., Frank, D., Fitzpatrick, G., Higgins, M.J., Tycko, B., 2004. Placental growth retardation due to loss of imprinting of Phlda2. Mech. Dev. 121, 1199–1210.
Saltzman, W., Maestripieri, D., 2011. The neuroendocrinology of primate maternal behavior. Prog. Neuro-psychopharmacol. Biol. Psychiat. 35, 1192–1204.
Scattoni, M.L., Crawley, J., Ricceri, L., 2009. Ultrasonic vocalizations: a tool for behavioural phenotyping of mouse models of neurodevelopmental disorders. Neurosci. Biobehav. Rev. 33, 508–515.
Schaller, F., Watrin, F., Sturny, R., Massacrier, A., Szepetowski, P., Muscatelli, F., 2010. A single postnatal injection of oxytocin rescues the lethal feeding behaviour in mouse newborns deficient for the imprinted Magel2 gene. Hum. Mol. Genet. 19, 4895–4905.
Scott, N., Prigge, M., Yizhar, O., Kimchi, T., 2015. A sexually dimorphic hypothalamic circuit controls maternal care and oxytocin secretion. Nature 525, 519–522.
Shingo, T., Gregg, C., Enwere, E., Fujikawa, H., Hassam, R., Geary, C., Cross, J.C., Weiss, S., 2003. Pregnancy-stimulated neurogenesis in the adult female forebrain mediated by prolactin. Science 299, 117–120.
Siegel, H.I., Rosenblatt, J.S., 1978. Duration of estrogen stimulation and progesterone inhibition of maternal behavior in pregnancy-terminated rats. Horm. Behav. 11, 12–19.
Simmons, D.G., Fortier, A.L., Cross, J.C., 2007. Diverse subtypes and developmental origins of trophoblast giant cells in the mouse placenta. Dev. Biol. 304, 567–578.
Simmons, D.G., Rawn, S., Davies, A., Hughes, M., Cross, J.C., 2008. Spatial and temporal expression of the 23 murine Prolactin/Placental Lactogen-related genes is not associated with their position in the locus. BMC Genom. 9, 352.
Smith, S.E., Zhou, Y.D., Zhang, G., Jin, Z., Stoppel, D.C., Anderson, M.P., 2011. Increased gene dosage of Ube3a results in autism traits and decreased glutamate synaptic transmission in mice. Sci. Transl. Med. 3, 103ra97.
Soares, M.J., 2004. The prolactin and growth hormone families: pregnancy-specific hormones/cytokines at the maternal-fetal interface. Reprod. Biol. Endocrinol. 2, 51.
Soares, M.J., Konno, T., Alam, S.M., 2007. The prolactin family: effectors of pregnancy-dependent adaptations. Trends Endocrinol. Metab. 18, 114–121.
Stohn, J.P., Martinez, M.E., Zafer, M., Lopez-Espindola, D., Keyes, L.M., Hernandez, A., 2018. Increased aggression and lack of maternal behavior in Dio3-deficient mice are associated with abnormalities in oxytocin and vasopressin systems. Genes Brain Behav. 17, 23–35.
Stolzenberg, D.S., Champagne, F.A., 2016. Hormonal and non-hormonal bases of maternal behavior: the role of experience and epigenetic mechanisms. Horm. Behav. 77, 204–210.
Surani, M.A., 1998. Imprinting and the initiation of gene silencing in the germ line. Cell 93, 309–312.
Suzuki, S., Shaw, G., Kaneko-Ishino, T., Ishino, F., Renfree, M.B., 2011. Characterisation of marsupial PHLDA2 reveals eutherian specific acquisition of imprinting. BMC Evol. Biol. 11, 244.
Suzuki, S., Shaw, G., Kaneko-Ishino, T., Ishino, F., Renfree, M.B., 2011. The evolution of mammalian genomic imprinting was accompanied by the acquisition of novel CpG islands. Genome Biol. Evol 3, 1276–1283.
Svare, B., Gandelman, R., 1976. Postpartum aggression in mice: the influence of suckling stimulation. Horm. Behav. 7, 407–416.
Svare, B., Mann, M., Samuels, O., 1980. Mice: suckling stimulation but not lactation important for maternal aggression. Behav. Neural Biol. 29, 453–462.
Swaney, W.T., Curley, J.P., Champagne, F.A., Keverne, E.B., 2008. The paternally expressed gene Peg3 regulates sexual experience-dependent preferences for estrous odors. Behav. Neurosci. 122, 963–973.
Takada, S., Paulsen, M., Tevendale, M., Tsai, C.E., Kelsey, G., Cattanach, B.M., Ferguson-Smith, A.C., 2002. Epigenetic analysis of the Dlk1-Gtl2 imprinted domain on mouse chromosome 12: implications for imprinting control from comparison with Igf2-H19. Hum. Mol. Genet. 11, 77–86.
Thiaville, M.M., Huang, J.M., Kim, H., Ekram, M.B., Roh, T.Y., Kim, J., 2013. DNA-binding motif and target genes of the imprinted transcription factor PEG3. Gene 512, 314–320.
Torner, L., Toschi, N., Pohlinger, A., Landgraf, R., Neumann, I.D., 2001. Anxiolytic and anti-stress effects of brain prolactin: improved efficacy of antisense targeting of the prolactin receptor by molecular modeling. J. Neurosci. 21, 3207–3214.
Torner, L., Toschi, N., Nava, G., Clapp, C., Neumann, I.D., 2002. Increased hypothalamic expression of prolactin in lactation: involvement in behavioural and neuroendocrine stress responses. Eur. J. Neurosci. 15, 1381–1389.
Trivers, R.L., 1974. Parent-offspring conflict. Integr. Comp. Biol. 14, 249–264.
Tsai, C.E., Lin, S.P., Ito, M., Takagi, N., Takada, S., Ferguson-Smith, A.C., 2002. Genomic imprinting contributes to thyroid hormone metabolism in the mouse embryo. Curr. Biol. 12, 1221–1226.
Tunster, S., Boque-Sastre, R., McNamara, G.I., Creeth, H.D.J., John, R.M., 2018. Sexually dimorphic response of the placental endocrine lineages to paternal loss of expression of Peg3. Front. Cell Dev. Biol. 6, 123.
Tunster, S.J., Tycko, B., John, R.M., 2010. The imprinted Phlda2 gene regulates extraembryonic energy stores. Mol. Cell. Biol. 30, 295–306.
Tunster, S.J., Van de Pette, M., John, R.M., 2011. Fetal overgrowth in the Cdkn1c mouse model of Beckwith-Wiedemann syndrome. Dis. Model Mech. 4, 814–821.
Tunster, S.J., Van De Pette, M., John, R.M., 2014. Isolating the role of elevated Phlda2 in asymmetric late fetal growth restriction in mice. Dis. Model. Mech. 7, 1185–1191.
Tunster, S.J., McNamara, G.I., Creeth, H.D., John, R.M., 2016. Increased dosage of the imprinted Ascl2 gene restrains two key endocrine lineages of the mouse Placenta. Dev. Biol. 418, 55–65.
Tunster, S.J., Creeth, H.D., John, R.M., 2016. The imprinted Phlda2 gene modulates a major endocrine compartment of the placenta to regulate placental demands for maternal resources. Dev. Biol. 409, 251–260.
Van De Pette, M., Tunster, S.J., McNamara, G.I., Shelkovnikova, T., Millership, S., Benson, L., Peirson, S., Christian, M., Vidal-Puig, A., John, R.M., 2016. Cdkn1c boosts the development of brown adipose tissue in a murine model of silver russell syndrome. PLoS Genet. 12, e1005916.
Vigod, S.N., Villegas, L., Dennis, C.L., Ross, L.E., 2010. Prevalence and risk factors for postpartum depression among women with preterm and low-birth-weight infants: a systematic review. BJOG 117, 540–550.
Walker, T.L., Vukovic, J., Koudijs, M.M., Blackmore, D.G., Mackay, E.W., Sykes, A.M., Overall, R.W., Hamlin, A.S., Bartlett, P.F., 2012. Prolactin stimulates precursor cells in the adult mouse hippocampus. PloS One 7, e44371.
Wohr, M., Schwarting, R.K., 2013. Affective communication in rodents: ultrasonic vocalizations as a tool for research on emotion and motivation. Cell Tissue Res. 354, 81–97.
Wu, Z., Autry, A.E., Bergan, J.F., Watabe-Uchida, M., Dulac, C.G., 2014. Galanin neurons in the medial preoptic area govern parental behaviour. Nature 509, 325–330.
Yamagata, T., Urano, H., Weeber, E.J., Nelson, D.L., Nishijima, I., 2008. Impaired hippocampal synaptic function in secretin deficient mice. Neuroscience 154, 1417–1422.
Zippelius, H.M., Schleidt, W.M., 1956. Ultraschall-Laute bei jungen Mäusen. Die Naturwiss. 43 502-502.