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Long-Term Alterations in Neural and Endocrine Processes Induced by Motherhood

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Abstract

The reproductive experience of pregnancy, lactation and motherhood can significantly remodel the female's biological state, affecting endocrine, neuroendocrine, neural, and immunological processes. The brain, pituitary gland, liver, thymus, and mammary tissue are among the structures that are modified by reproductive experience. The present review that focuses on rodent research, but also includes pertinent studies in sheep and other species, identifies specific changes in these processes brought about by the biological states of pregnancy, parturition, and lactation and how the components of reproductive experience contribute to the remodeling of the maternal brain and organ systems. Findings indicate that prior parity alters key circulating hormone levels and neural receptor gene expression. Moreover, reproductive experience results in modifications in neural processes and glial support. The possible role of pregnancy-induced neurogenesis is considered in the context of neuroplasticity and behavior, and the effects of reproductive experience on maternal memory, i.e. the retention of maternal behavior, together with anxiety and learning are presented. Together, these sets of findings support the concept that the neural and biological state of the adult female is significantly and dramatically altered on a long-term basis by the experiences of parity and motherhood. Remodeling of the maternal brain and other biological systems is posited to help facilitate adaptations to environmental/ecological challenges as the female raises young and ages.

Keywords

Behavior; Endocrine; Neuroendocrine; Parity; Prolactin; Reproductive Experience

A myriad of developmental experiences forge the operations of the adult female brain. These experiences include the female's prenatal and postnatal environments, early life experiences, nutrition, puberty, pregnancy, lactation, and social interactions throughout her lifetime. Recent research primarily conducted in rodents and to a lesser extent in sheep, women and other mammals, has established that during adulthood the "reproductive experience" that comprises pregnancy, lactation and motherhood can profoundly affect her

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physiology and behavior on a long-term, if not permanent, basis. Changes brought about by pregnancy and lactation contribute to alterations in a number of neural and biological systems, including key neuroendocrine and endocrine processes. The present review focuses upon the specific long-term shifts in neural, endocrine, immune and behavioral processes that result from the reproductive experience that accompanies motherhood. In addition to examination of the neurobiology of motherhood following pregnancy and lactation, the consequences of maternal care itself, independent of pregnancy and lactation, are explored, and comparisons made between these processes in females undergoing single or multiple pregnancies and lactations with those of non-parous maternal and non-maternal females. When taken together, these examinations reveal that prior motherhood, especially following a pregnancy and lactational period, results in demonstrable long-term alterations in neuroanatomical, neurochemical, neuroendocrine, endocrine, immunological and behavioral systems that are adaptive to the adult female in promoting species propagation and survival.

Reproductive Experience

Adult female mammals can undergo a range of physiological changes associated with their reproductive experiences. The term reproductive experience here is meant to include both the singular events of mating, pregnancy, parturition, and lactation as well as the combination of these events. Moreover, since females can give birth once or multiple times as well as not give birth, the term multiple reproductive experiences is designated for so-called multiparous females (see Table 1 for classification of reproductive histories).

The reproductive status of the female as it turns out can significantly impact both her physiological and neurobiological states as reflected by differences in hormonal, neurotransmitter, neurochemical, neuroanatomical and behavioral states. The present paper specifically examines the plasticity induced by motherhood and the changes associated with and produced by these various reproductive experiences (see Figure 1).

Reproductive Experience and Endocrine Functions

One of the first studies documenting long-term changes in hormone levels resulting from reproductive experience was reported in women (Musey et al., 1987). Circulating prolactin (PRL) levels were significantly lower during the follicular phase of the menstrual cycle in previously parous women up to 10 years following giving birth to a child. Similar reductions in blood androgens and estrogens were detected when hormone levels in previously parous women were compared with those of nulliparous, age-matched subjects (Musey et al., 1986; 1987). A similar pattern of reduced PRL blood concentrations was subsequently identified in pregnant rats when PRL levels were compared between rats undergoing a second pregnancy (gestation day 12) with those found during a first pregnancy (Bridges and Hammer, 1992). Examination of circulating PRL levels during the mating-induced diurnal and nocturnal PRL surges in primigravid and multigravid rats, likewise, found reductions in PRL levels during these surge periods (Bridges et al., 1993; see Figure 2) and shifts in hypothalamic monoaminergic activity in the multigravid subjects (Sider et al., 2002).

Similar reductions in circulating PRL were also found when PRL levels were compared between rats that experienced a prior parity with those of age-matched, nulliparous females;

PRL levels were lower in cycling primiparous subjects on proestrus of the estrous cycle (Bridges & Byrnes, 2006) as well as on diestrus (Anderson et al., 2006). Moreover, the suckling stimulus provided by young which induces a characteristic increase in circulating PRL induces a smaller increase in plasma PRL in multiparous (2nd lactation) mother rats when compared with that induced by suckling in primiparous dams (Mann and Bridges, 1992). This experiential shift in endocrine responsivity, i.e. reduced PRL secretion, likely involves neuroendocrine factors, including dopamine and endogenous opioids, which regulate PRL release during lactation (Ben-Jonathan, 1985; Selmánoff and Gregerson, 1986). The physiological consequences of this overall dampening of PRL secretion may reflect a shift in endocrine efficiencies such that less hormone may be needed to stimulate endocrine responses, such as lactogenesis, corpora lutea maintenance, and perhaps the female's behavior. Evidence for a shift in PRL responsiveness is presented later in this discussion.

Prior reproductive experience also affects plasma estrogen levels during subsequent estrous cycles in rats; overall estradiol-17 β are reduced in cycling primiparous compared with that of nulliparous controls (Bridges and Byrnes, 2006). The modest reduction in estrogen levels may partially account for the smaller estrogen-stimulated proestrus luteinizing hormone surge in previously parous rats (Bridges, unpublished data) and contribute to the prolongation of estrous cyclicity with reproductive experience over the female's lifetime by some undetermined mechanism (Matt et al., 1987; Hopwood et al. 1998). Estrogen-induced PRL sensitivity also shifts as a function of reproductive experience. Gonadectomized nulliparous rats were more sensitive than multiparous rats to lower doses of estradiol benzoate (EB), whereas multiparous subjects were more responsive to a high dose of EB (Bridges and Byrnes, 2006). Hussain et al. (2013) found a similar enhanced sensitivity of nulliparous rats to estrogen when examining response task learning and memory; this effect was not found in non-lactating, primiparous females. Thus, parity-induced alterations in the female's endocrine state that persists beyond lactation may variably impact estrogen-mediated processes over the female's life span.

An examination of possible alterations in glucocorticoid activity as a function of reproductive experience revealed that serum corticosterone concentrations during early lactation in rats were significantly higher in primiparous than in multiparous dams (Pawluski et al., 2009). As lactation progressed, this difference disappeared. Since glucocorticoids are essential as part of the lactogenic complex of hormones, one can speculate that the higher levels of corticosterone may function to establish the initial lactation, a process that becomes more efficiently activated by endocrine factors during subsequent lactations.

Reproductive experience induced changes in endocrine system are not limited to the pituitary--gonadal axis, but also extend to other endocrine target tissues including the mammary glands and liver. In the rat increased expression of estrogen receptor β 2 is found in mammary tissue as a function of prior parity (Kass et al., 2004). Likewise, the number of births affects expression of the long form of the PRL receptor gene in rat mammary tissue (Bridges et al., 2011). *PRLr* gene expression increases in mammary tissue of primiparous versus age-matched, multiparous rats, a pattern opposite that found in neural tissues (Anderson et al., 2006). In contrast, in hepatic tissue *PRLr* gene expression was increased in

primiparous mothers compared with that in multiparous dams (Bridges et al., 2011). These shifts in PRL receptor expression in mammary and hepatic tissues may reflect alterations in hormone sensitivity that enhance lactogenesis along with altered metabolic activity in multiparous dams.

Reproductive Experience Induced Neurochemical Changes

In addition to the above noted alterations in hormone release that develop as a consequence of prior reproductive experience, long-term changes in neurochemical activity also result from the prior experience of motherhood. Some of these changes appear to contribute to the shifts in endocrine activity, while others influence independent neural events that affect processes such as maternal care, memory, and arousal.

One of the more profound shifts in neurochemical activity induced by reproductive experience involves what appears to be a general up-regulation or enhanced activity of the neural dopaminergic systems. Early studies demonstrated that the reproductive experience of pregnancy plus lactation in rats rendered experienced dams less responsive to the ability of haloperidol, a dopamine antagonist, to induce PRL secretion (Bridges et al., 1997; see Figure 3). This effect was only present when the subjects had gone through both a prior pregnancy and lactation, since animals experiencing only pregnancy, but no lactation, responded similarly to nulliparous animals (Byrnes and Bridges, 2005). Thus, some postpartum lactational and/or behavioral interactions with young are needed to modify the subsequent shift in sensitivity to haloperidol. In women, a similar shift in PRL-induced sensitivity develops as a result of motherhood (De los Monteros et al., 1991). When challenged with orally administered metoclopramide, a dopamine antagonist, women who had given birth and nursed their babies were found to release less PRL than comparable treated nulliparous women. When taken together, these findings indicate the development of a reproductive experience-enhanced inhibitory dopamine tone occurs within the tuberoinfundibular-pituitary axis, a shift that is reflected in dampened pituitary PRL secretion. This reproductive experience-induced shift in dopamine regulated PRL secretion may also involve a possible modification in the actions of dopamine at the level of the adenohypophysis (Byrnes and Bridges, 2007). An examination of dopamine D₂ receptor mRNA in the anterior pituitary gland during the estrous cycle of previously primiparous rats with that of nulliparous subjects revealed an increase in D_{2 long} mRNA on proestrus together with a decrease in D_{2 short} mRNA in the experienced animals. Since the ratio of D_{2 long} to D_{2 short} can affect lactotroph proliferation and PRL secretion, a shift in relative expression of the two D₂ receptor isoforms within the adenohypophysis of previously parous subjects may contribute to the reduction in PRL secretion that occurs in experienced females.

Changes in dopamine release and activity as a function of reproductive experience are also evident within the nigrostriatal dopamine system (Hucke et al., 1998; 2001; Felicio et al., 2006). Measurements of dopamine and its metabolite DOPAC in the striatum of intact multiparous and nulliparous rats indicated significantly higher levels of DOPAC in multiparous animals as well as a strong trend towards higher levels of dopamine ($P < 0.08$) when compared with levels in nulliparous females (Byrnes et al., 2001). Examination of dopamine and DOPAC levels in the nucleus accumbens shell region of the mesolimbic

dopamine system in these same subjects failed to detect differences as a function of reproductive history. During pregnancy, however, rats undergoing a second pregnancy have higher concentrations of both DA and DOPAC in both the striatum and hypothalamus (Hucke et al., 1998). Hence, these neural changes may be more localized within the striatum and hypothalamus. The increased dopaminergic activity in the hypothalamus produced by reproductive experience may provide greater inhibition of PRL release and thereby contribute to the dampened PRL surges found in multigravid rats (Bridges and Hammer, 1992; Bridges et al., 1993), whereas the established increases in DA and DOPAC concentrations in these other neural regions may impact the female's behavioral and related neural responses.

One such behavioral response that assesses dopamine activity is prepulse inhibition (PPI) following an apomorphine challenge. PPI is the reduction in startle amplitude that is found when a weak acoustic stimulus precedes the presentation of a startle-eliciting stimulus (Graham, 1975; Byrnes et al., 2001). Comparisons of both PPI and stereotypy as a function of reproductive experience in rats indicated that forebrain dopamine receptors are more sensitive in subjects that have experienced pregnancy and lactation when compared to responses measured in nulliparous females (Byrnes et al., 2001). Nulliparous rats were less responsive to the dopamine agonist apomorphine in the PPI test than age-matched, non-lactating females with prior reproductive experience. Moreover, experienced females displayed enhanced responsiveness to apomorphine-induced oral stereotypy (see Figure 4). Analyses of DOPAC in micropunches taken from the dorsolateral striatum revealed higher levels of DOPAC in intact, diestrous, multiparous versus nulliparous females. In a related study involving the measurement of dopamine release in the nucleus accumbens shell (NAs) in response to pup stimuli, Afonso et al. (2008) found that rats with prior maternal experience (whether nulliparous or multiparous) displayed increased dopamine release during the initial 8 minutes of pup exposure, whereas inexperienced nulliparous rats failed to display any increase in dopamine release. These findings appear to reflect normative developmental changes in female brain functions brought about by reproductive experience and support the concept that broad changes in dopamine function affecting endocrine, neuroendocrine and behavioral processes emerge following the experience of pregnancy and lactation.

Related studies in sheep have demonstrated that reproductive experience alters other neurochemical processes. Specifically, retrodialysis of either oxytocin or vasopressin into the olfactory bulbs of multiparous and nulliparous ewes found that both peptides induced a greater increase in acetylcholine and noradrenaline in multiparous subjects (Lévy et al., 1995). Furthermore, glutamate and GABA release was significantly increased in the olfactory bulb at parturition in multiparous, but not primiparous ewes (Keverne et al., 1993). Moreover, a potassium challenge produced a greater increase in glutamate and GABA release in multiparous than nulliparous ewes. Together, these studies demonstrate that transmitter release in the olfactory bulb, a structure critical for the establishment of maternal behavior in the ewe, is affected by the female's reproductive history (Lévy and Keller, 2008).

Although less thoroughly studied, the endogenous opioid system also undergoes modifications as a function of reproductive experience. The initial landmark study that identified an effect of reproductive experience examined opiate regulation of rat maternal behavior (Kinsley and Bridges, 1988). It was found that the ability of morphine to disrupt ongoing maternal care (Bridges and Grimm, 1982) was significantly attenuated in lactating rats during a second lactation when compared with the disruptive effects of morphine present in age-matched, primiparous, lactating mothers (Kinsley and Bridges, 1988). Since opiates and their receptors are present in the medial preoptic area (MPOA) and this neural region is a key regulatory site for maternal care (Numan, 1974, Numan et al., 1977), we then characterized the status of MPOA mu-opiate receptors by immunocytochemistry as a function of reproductive experience (Bridges and Hammer, 1992). Shifts in the densities of mu-opiate receptors were present in the MPOA during both pregnancy (primigravid versus multigravid) and lactation (first versus second). Notably, MPOA opiate receptor densities were higher in multiparous rats on day 5 of lactation when compared with those of primiparous subjects. Moreover, while the densities decreased between pregnancy and lactation in primiparous rats, densities remained unchanged across pregnancy and lactation in multiparous subjects. How these experiential-induced changes in mu-opiate receptor densities impact the female's physiology and behavior is not known. However, the shift in receptor patterns may be associated with changes in behavioral sensitivities to opiates. In a follow-up study, the effects of MPOA infusions of beta-endorphin on maternal behavior were compared between age-matched, lactating primiparous and multiparous rats (Mann and Bridges, 1992). As is the case with systemic morphine treatment, the ability of central beta-endorphin to disrupt maternal care was dramatically reduced in multiparous mothers (see Figure 5). Hence, reproductive experience renders the MPOA of lactating rats less responsive to opiates in the presence of higher densities of mu-opiate receptor densities. It is unknown whether this change persists beyond the physiological state of lactation into later adult life. Another neural region where opiate receptor activity is affected by reproductive experience is the periaqueductal gray (PAG). Expression of both the mu and kappa opiate receptors increase in rats with multiple reproductive experiences (Teodorov et al., 2011). It has been proposed that such changes may underlie improved foraging behaviors as well as maternal care in the more experienced dam (Sukikara et al., 2006; Mota-Ortiz et al., 2009).

A more recent microarray study that examined the effects of parity and maternal care on gene expression detected an up-regulation of dopamine-related genes in the MPOA together with shifts in the mu-opioid gene (OPRM1) and the GABA receptor gene (Akbari et al., 2013). In the medial amygdala (mAMY), increased expression of genes in the glucocorticoid family (CYP11B1a) was detected. These shifts in gene expression may provide a modified substrate that helps account for the effects of reproductive experience on opioid and dopamine actions in neural structures such as the MPOA and amygdala that are key players in the regulation of maternal behavior and other neural processes.

Another important avenue of research has explored the possible effects of reproductive experience on the neural prolactin receptor system (Anderson et al., 2006; Sjöholm et al., 2011). Since circulating PRL levels are generally reduced in experience females and PRL acts directly on the MPOA, presumably through PRL receptors, to stimulate maternal behavior (Bridges et al., 1990; Bridges et al., 2001), it was asked whether PRL receptor

expression and its regulation within the MPOA of the female rat might change as a function of reproductive experience. Expression of the long form of the PRL receptor was compared between age-matched, nulliparous and non-lactating, primiparous subjects on diestrus of the estrous cycle. The primiparous rats had given birth and raised a litter to weaning 21 days later. Eight weeks later sets of both treatment groups were injected subcutaneously with ovine PRL or vehicle and brains were subsequently processed for PRL receptor (long form), suppressor of cytokine signaling (SOCS)-1 and SOCS-3 in the MPOA, paraventricular nucleus (PVN) and arcuate nucleus (ARC). As shown in Figure 6 (left panel), the long form of PRL receptor mRNA in the MPOA and ARC were elevated by reproductive experience. Moreover, after PRL injections, PRL receptor expression in these regions increased in reproductively experienced females, but was without effect in virgin subjects (Anderson et al, 2006). Thus, reproductive experience renders the MPOA and ARC more sensitive to PRL exposure as reflected by enhanced PRL receptor expression. Similar changes in sensitivity to PRL were found in SOCS1 and SOCS3 mRNA levels in reproductively experienced rats, reflecting a shift in the modulation of PRL receptor activity. In a later study, it was shown by immunohistochemistry (see Figure 6, right panel) that the number of immunoreactive cells containing phosphorylated STAT5 (pSTAT5), which mediates PRL receptor actions, increases in the MPOA, paraventricular nucleus, supraoptic nucleus, and ARC after reproductive experience, whereas pERK1/2 activity is unaffected (Sjoholm et al., 2009). In addition, reproductive experience increased kisspeptin neuron pSTAT5 co-localization after intraventricular PRL treatment in reproductively experienced rats. Thus, STAT5 activity, which mediates PRL/PRL receptor actions within neurons, is enhanced by reproductive experience in multiple hypothalamic regions.

Reproductive experience also alters estrogen receptor status in the female brain. In rats, prior reproductive experience increased the number of estrogen receptor alpha (ER α) cells during the afternoon of proestrus in the anterior-dorsal MPOA and decreases immunoreactive ER α cell numbers in the anterior medial amygdala at the same time (Byrnes et al., 2009). In sheep, estrogen receptor alpha cell density is increased during a second pregnancy relative to a first pregnancy (Meurisse et al., 2005) with changes found during late pregnancy in the MPOA, PVN, SON and mAMY. Given the important role for estrogens in maternal behavior (Bridges, 2015) and the recent finding that PRL treatment enhances STAT5 activity in ER α neurons in the female mouse brain (Furigo et al., 2014), the relationships between alterations in neural sensitivity to PRL and the apparent shift in estrogen receptor activity may play an important role in regulating enhancements of maternal care, i.e. the retention of maternal behavior, long after the cessation of lactation. Note that the neurobiological consequences of parity on maternal behavior are discussed in greater detail later in this article.

Apart from the changes in endocrine-related mediators associated with the regulation of maternal behavior, reproductive experience affects the number of orexin-A-immunoreactive (ORA-ir) cells in the prairie vole (Donlin et al., 2014). The total number of OXA-ir cells were greater in the anterior caudal hypothalamus in experienced females than those found in control females. Given that orexin cells integrate a number of signals that affect mood and emotion as well as pair bonding and perhaps lactation (Sellayah and Sikder, 2103; Tsujino

and Sakurai, 2013), the possible role of these reproductive experience-induced changes merits further investigation.

Reproductive Experience Induced Neuroanatomical Changes

Reproductive experience results in short and long-term neuroanatomical changes. The short-term effects of parity during the postpartum period were first described by Hatton and Tweedle (1982) who reported increases in glial withdrawal in the supraoptic nucleus in parturient rats and increases in neuronal connectivity. These alterations, however, were not permanent as restructuring of this part of the hypothalamus involved in oxytocin production and release reverted to the virgin status following weaning. More recently, evidence in support of long-term alterations in neuronal connectivity has emerged. Featherstone et al., (2000) found that in rats reproductive experience has longer term effects on neural substrates through the alteration of brain astrocyte numbers. Specifically, when day 5 postpartum multiparous rats were given postpartum contact with pups, a higher number of glial fibrillary acidic protein (GFAP) positive cells were identified in the MPOA when compared with numbers in day 5 lactating primiparous dams. GFAP cell numbers, in contrast were reduced as a function of multiparity in the medial amygdala and habenula region. Modifications in the neural connectivity as a result of maternal experience have also been reported to occur within the MPOA (Shams et al., 2012). The neuronal complexity within the MPOA is altered as reflected by increases in primary dendritic branching and reductions in higher order branching. These changes may help account for longer term changes in both behavioral and neuroendocrine processes that develop with reproductive experience.

Although less implicated directly in the maternal neural network, studies of hippocampal anatomy have also revealed that reproductive experience alters cell proliferation and cell survival in the dentate gyrus of the hippocampus (Pawluski and Galea, 2007). During the early postpartum period there is a decline in cell proliferation in this region in both primiparous and multiparous rats accompanied by a decrease in cell survival in primiparous, but no change in cell survival in multiparous subjects. Moreover, pyramidal neurons in the CA1 and CA3 regions of the hippocampus exhibit greater dendritic pruning in primiparous dams at the time of weaning compared with that found in multiparous rats (Pawluski and Galea, 2006). In a related study, Kinsley et al. (2006) reported increases in dendritic spines in the CA1 region of the hippocampus during late pregnancy and early postpartum, an effect presumably brought about by changes in hormone exposure during gestation. Furthermore, when both virgin and maternal rats were injected with the neural toxin, kainic acid (KA), and GFAP was assessed in the hippocampus, the GFAP immunoreactivity was higher in the virgin subjects, indicating that the experience of pregnancy and lactation may provide some protection against KA insult (Franssen et al., 2012). The higher level of GFAP activity in virgins may reflect more extensive KA-induced damage produced in the CA1 region of the hippocampus and hence further alterations in the neurobiological state produced by reproductive experience. Again, it is not known the permanency of such changes, a question still to be resolved.

Possibly serving as a substrate for neuroplastic changes in the maternal brain, the processes of cell proliferation and neurogenesis are interesting candidates as mediators of both

neurobiological and behavioral changes in the experienced female. Two established regions of the brain where new neurons are produced are the subventricular zone (SVZ) and hippocampus. Most notably, neuroblasts produced within the SVZ migrate via the rostral migratory stream to the olfactory bulb where they become incorporated as neurons into the olfactory system, thereby modifying existing connectivity (Shingo et al., 2004). During pregnancy in rodents neurogenesis and cell proliferation in the SVZ are enhanced (Shingo et al., 2004; Furuta and Bridges, 2009; Larsen and Grattan, 2010). While the role of pregnancy-induced neurogenesis remains to be firmly established, in mice it appears to be stimulated by the hormone prolactin (Shingo et al., 2004) and helps reduce postpartum anxiety and depression (Larsen and Grattan, 2010). In contrast to what has been found in rodents, in sheep cell proliferation and neurogenesis during pregnancy and at birth actually decline (Brus et al., 2010). Brus and colleagues hypothesized that the postpartum learning of maternal behavior in sheep, i.e. lamb acceptance and identification, may actually be associated with a down-regulation in olfactory neurogenesis and an enhancement of olfactory neuroblast maturation (Brus et al., 2014; Lévy, et al., 2011). These investigators proposed that the down regulation of cell proliferation in the early postpartum period in sheep might facilitate olfactory perceptual and memory demands, promoting bonding and the survival and integration of neurons born earlier. Since the pattern of olfactory and hippocampal neurogenesis in adult sheep appears similar to that in humans and differs from that found in rodent animal models (Brus et al., 2013), these researchers suggested that sheep may in fact provide a better translational model for neurogenesis in humans.

Immune Function and Reproductive Experience

The female's prior reproductive history also appears to affect her subsequent immunological status (Carvalho-Freitas et al., 2007; 2011; 2013). It appears that changes in neuroendocrine functions, specifically shifts in PRL secretion and sensitivity, underlie enhanced macrophage activity with increased reproductive experience. In female rats tested for macrophage activity two to three weeks after weaning their young, phorbol miristate acetate (PMA)-induced oxidative burst was decreased in peritoneal macrophages from primiparous rats when compared with that in nulliparous subjects (Carvalho-Freitas et al., 2007). This change appears to be associated with circulating prolactin concentrations as suppression of PRL with bromocriptine increases macrophage mortality and impairs lymphocyte proliferation after exposure to *Listeria monocytogenes* (Bernton et al., 1988). It is noted that the decreased PRL secretion is characteristically found following parity in both rats and women (Musey et al., 1987; Bridges and Hammer, 1992; Bridges et al., 1993; Bridges and Byrnes, 2006; Anderson et al., 2006). This decline is associated with an increase in dopaminergic tone (Bridges et al., 1997; Huckle et al., 2001; Carvalho-Freitas et al., 2007). Comparisons of the ability of PRL to induce macrophage activity in primiparous and multiparous lactating rats indicated that macrophages obtained from peritoneal fluid of multiparous rats that have reduced circulating PRL and greater dopaminergic activity are more sensitive to PRL in terms of oxidative bursts and phagocytosis than that present in fluid obtained from primiparous lactating rats (Carvalho-Freitas et al., 2011). This increased sensitivity to PRL is similar to the increased neural sensitivity to PRL's ability to induce neural PRL receptor expression (Anderson et al., 2006). Likewise, peritoneal fluid obtained

from primigravid and multigravid rats from days 17–21 of pregnancy and tested for macrophage induction after PRL treatment found that *in vitro* macrophages from the multigravid rats responded more effectively to PRL (Carvalho-Freitas et al., 2013). Thus, reproductive experience, possibly through changes in neuroendocrine activity, modify the female's immune responses. It is unknown whether similar changes in immune function occur in other mammals, but the potential interactions between the nervous and immunological systems provides an important window to view the impact of reproductive experience on disease susceptibility.

Behavioral Consequences of Reproductive Experience

The experiences of pregnancy and lactation can exert long-lasting effects on a range of behaviors that extend well into the female's adult life. Some of the more pronounced changes include shifts in maternal behavior, anxiety, and learning. These changes are noted here.

Reproductive Experience and Maternal Behavior

Reproductive experience can profoundly affect the female's subsequent responsiveness to young. The most comprehensive studies on the effects of prior parity on maternal care have been conducted in rats, although similar findings of experience on maternal performance have been reported in sheep, primates, and women (see Fleming et al., 2008; MacBeth and Luine, 2010; Poindron and Neindre, 1980; Ruppenthal et al., 1978).

Prior maternal experience affects the subsequent expression of maternal care well beyond the end of lactation and prior maternal-young interactions (Moltz & Robbins, 1965; Moltz et al., 1966; Bridges, 1975). Studies of the long-term effects of parity on maternal behavior in later adulthood in rats demonstrated that a minimal of exposure postpartum in a primiparous rat is sufficient to result in shortened maternal induction latencies, i.e. maternal memory, 25 days postpartum after last exposure to young (Bridges, 1975; see Figure 7). When primiparous rats were allowed to interact with their newborns for 4–6 hours postpartum after which their litters were removed, they displayed response latencies averaging one day when tested for maternal behavior with foster young 25 days later, a response similar to that of mothers given 21 days of maternal experience. Both of these groups responded significantly faster than did virgin females or primigravid rats caesarean-sectioned and given no post-surgical pup exposure. Parturition itself does not appear essential for the establishment of this long term retention of maternal care as rats caesarean sectioned a day prior to expected parturition and then exposed to newborn pups for a brief period after surgery displayed a rapid induction of maternal care towards foster young 25 days later (Bridges, 1977). Fleming and colleagues have systematically investigated the neural basis of maternal memory in rats (Orpen and Fleming, 1987; Fleming et al., 1990; Lee et al., 1999; Li and Fleming, 2003a; Li and Fleming, 2003b). These investigators found that the formation of maternal memory postpartum appeared to be dependent upon protein synthesis as dams treated with cycloheximide did not display a full retention response (Fleming et al., 1990). Moreover, a crucial role for the shell region of nucleus accumbens was demonstrated (Li and Fleming, 2003a). Bilateral electrolytic lesions of the NAs, but not core region, around the time of birth resulted in a significant disruption of reinduction latencies 10 days later (Li and

Fleming, 2003a). Furthermore, infusions of cycloheximide into the NAc an hour after birth disrupted maternal memory 10 days later; similar infusions into the MPOA was without effect, indicating that protein synthesis in this dopamine sensitive structure around the time of parturition mediated the establishment of maternal memory (Li and Fleming, 2003b).

Whereas the DA D1 receptor within the NAc more recently was shown to play a clear role in stimulating the initial onset of maternal behavior in the rat (Stolzenberg et al., 2007), earlier experiments using pharmacological approaches identified a role for the dopamine D1 and D2 receptors in maternal memory (Byrnes et al., 2002; Parada et al., 2008). The initial study reported a role for the DA D2 receptor in maternal memory using systemically administered drugs (Byrnes et al., 2002), but did not identify a role for the DA D1 receptor in maternal memory. A more recent study, however, in which DA D1, DA D2, or mixed antagonists were directly infused into the shell region of the nucleus accumbens identified a role for both the DA D2 as well as the DA D1 receptor within the NAc in the consolidation of maternal memory (Parada et al., 2008). Thus, activation of the mesolimbic DA system and its actions at the level of the NAc promote both the initial induction of maternal behavior in the rat together with the establishment of maternal memory.

Opiates during the periparturitional period, like dopamine, appear to facilitate the establishment of maternal memory (Byrnes and Bridges, 2000). Treatment of pregnant rats with the long-acting mu-opioid receptor antagonist, beta-funaltrexamine, prior to parturition interferes with the establishment of maternal memory assessed seven days later, whereas treatment with the same compound 24 hours postpartum failed to disrupt the retention of maternal memory. These findings indicate that opiate activity around the time of parturition may also affect the subsequent expression of maternal memory. The possible interactions of neural dopamine and opioid systems in maternal memory is a topic of future inquiry.

Oxytocin activity is also affected by reproductive experience. In mice, an enhanced level of maternal responsiveness is associated with increased oxytocin levels (Lopatina et al., 2011). The glycoprotein, CD38, has been found to play a role in social behavior through its stimulation of oxytocin (Jin et al., 2007). When parental behaviors were compared between primiparous and multiparous wild-type and CD knockout mice, faster retrieval responses were found in multiparous than primiparous CD38 knockout dams (Lopatina et al., 2011). Moreover, response latencies were faster in wild-type multiparous as compared with primiparous mothers. In rats, infusions of an oxytocin antagonist into the nucleus accumbens shell region 1 hour after birth was shown to interfere with the expression of maternal memory after a 10-day pup isolation period. Infusion of oxytocin itself using a similar model, however, did not potentiate maternal memory (D'Cunha et al., 2011). In sheep, oxytocin release in the olfactory bulb of parturient ewes is greater in multiparous than primiparous ewes (Lévy et al., 1995). These findings indicate that oxytocin activity and responsiveness is altered by reproductive experience and thus may contribute to the enhancement of maternal care produced by reproductive experience.

Overall, the changes in maternal responsiveness and/or maternal motivation induced by motherhood following birth appear to be potentiated by the reproductive experiences of pregnancy and parturition. Whereas maternal experience in virgin female rats (Rosenblatt,

1967) results in enhanced maternal responsiveness later in life (Cohen and Bridges, 1981; Scanlan et al., 2006), the intensity of responsiveness in previously parous subjects is significantly shorter than that of previously pup-induced maternal virgin rats up to three months following an initial maternal experience (Scanlan et al., 2006). Moreover, when the responses to pup presence is tested on an elevated plus maze, previously parous rats that were re-induced to display maternal care display enhancements of both time spent on the open arms and distance traveled relative to reinduced maternal nulliparous subjects (see Figure 8). Furthermore, when both c-Fos cell numbers in designed brain regions are counted in re-induced virgin and parous subjects after exposure to non-tactile pup cues on an elevated plus maze (EPM) apparatus, more c-Fos-labeled cells were found in the cortical and medial nuclei of the amygdala in previously parous dams exposed to pups, but no changes were apparent in the re-induced virgin subjects (Scanlan et al., 2006). Comparisons of Fos-like immunoreactivity (Fos-Lir), likewise, revealed changes in brain chemistry as a function of parity (Fleming and Korsmit, 1996). Fos-Lir in response to pup stimuli is enhanced in females following parity. Specifically, rats that give birth and interact with young for a few hours postpartum exhibit increased Fos-Lir in the MPOA, basolateral AMY, and both the parietal and prefrontal cortex when dams were exposed to pups ten days later (Fleming and Korsmit, 1996). Since Fos activity is thought to reflect neuronal activity, these findings support the idea that the underlying neural substrate that regulates maternal behavior becomes more reactive and sensitized to sensory cues with increasing reproductive experience. It appears that the full bio-behavioral experience of pregnancy, parturition, and maternal behavior results in long-term shifts in the reward valences to cues from young as reflected by changes in how the brain responds to such stimuli.

In addition to changes in pup-oriented responses induced by a prior parity, another important aspect of maternal care, that is maternal defense or aggression, is also modified by parity. Specifically, multiparous lactating rats initiate attacks towards an intruder male faster than do age-matched, primiparous lactating dams during early lactation (day 5). In addition, the number of attacks exhibited by the multiparous mothers are increased as are the cumulative duration of attacks (Nephew et al., 2009). The enhanced levels of maternal aggression in the more experienced mothers appears to be regulated in part by the neuropeptide arginine vasopressin (AVP) as central infusions of AVP reduced the levels of maternal aggression in multiparous dams on lactation day 15 (Nephew et al., 2010). Hence, both pup-oriented and protective responses are altered by parity or multiparity.

It is also noteworthy that the effects of reproductive experience on maternal care are not limited to rodents, but are found across a number of mammalian species. In sheep, multiparous ewes are more likely to adopt an alien lamb than either nulliparous or primiparous ewes (Lévy and Poindron, 1987). Similar higher levels of maternal care are likely in sows as they care for their piglets. In non-human primates, repeated births are accompanied by an increase in caregiving and a decrease in neglect and abusive behaviors (Ruppenthal et al., 1976). In women, whereas the effects of parenthood on neural activation has received recent attention, most of the studies have focused on the activation of neural regions during active motherhood and less so on the potential long-term changes in neural activation associated with a prior parity (Strathearn, 2011; Kim et al., 2014). However, in a couple of studies using fMRI the effects of prior reproductive experience have been

measured to examine possible changes in neural activation between first time and experienced mothers (Swain et al., 2007; 2011). Whereas the effects of multiparity, like a single birth, on maternal care appear to be similar, greater neural activation was evident in the more experienced mothers.

Reproductive Experience and Anxiety

Motherhood not only brings about pronounced shifts in anxiety (Lonstein, 2007) and stress responsivity (Brunton and Russell, 2008; Brunton, 2010) during pregnancy and lactation, but anxiety levels in females are modified well into later adulthood by prior reproductive experience (Byrnes and Bridges, 2006; Byrnes et al., 2012). In an initial study, anxiety was measured in female rats with varied reproductive histories on the elevated plus maze (EPM) and in an open-field test (Byrnes and Bridges, 2006). In age-matched nulliparous and non-lactating primiparous rats tested on proestrus, anxiety levels measured on the EPM were attenuated in the experienced subjects relative to that of nulliparous controls (see Figure 9). A similar pattern of responses was found in the open field test. When the effects of prior reproductive experience were examined in later adulthood, i.e. middle age, parity was found to be associated with increased anxiety. One might consider this the “grandmother” effect. Interestingly, the effects of reproductive experience on the EPM were eliminated in both age groups by gonadectomy.

In order to clarify the possible role of ovarian hormones, namely estrogens, in this process, a second set of studies were conducted. Previous research had indicated a role for ER β in regulating anxiety in rats (Lund et al., 2005). However, our interest turned more to the possible role of ER α given that reproductive experience results in increased immunoreactive ER α cell numbers in selected brain regions in female rats (Byrnes et al., 2009). We therefore evaluated the possible involvements of both ER α and ER β in reproductive experience-induced shifts in anxiety (Byrnes et al., 2012). Treatment of reproductively experienced rats with the ER α agonist PPT or the ER β agonist DPN differentially affected EPM responses. Where PPT increased time spent in the open arms in non-lactating primiparous rats, the ER β agonist, DPN, did not affect anxiety in either primiparous and nulliparous subjects. Furthermore, reproductive experience and PPT treatment significantly altered corticotropin releasing hormone (CRH) mRNA gene expression in the paraventricular nucleus and amygdala. These findings indicate that reproductive experience renders the female more responsive to ER α -mediated reductions in anxiety and alters central stress-related CRH gene activity.

Reproductive Experience and Other Behavioral Modifications

Studies of salt intake as well as learning are also affected by the female’s prior reproductive history. In a study examining the effects of a prior parity on salt intake, it was found that the increased salt intake that develops over pregnancy and lactation persisted beyond the cessation of lactation (Frankmann et al., 1991). When dams were sodium depleted following their reproductive episodes, the need-induced sodium chloride intake was significantly increased compared to that of virgin rats. This shift in salt intake may reflect changes in vasopressin-mediated renal regulation by the hypothalamus. The specific mechanisms that regulate this change in salt need remains unknown.

In addition to this behavioral shift, prior reproductive experience also alters learning performance (Pawluski et al., 2006; Kinsley and Lambert, 2008; Macbeth and Luine, 2010). For example, when rats with varying reproductive histories are tested for performance on a dry learning maze, higher levels of responses, i.e. shorter latencies, to find the baited food well are found in previously parous subjects from 6 to 24 months of age (Kinsley and Lambert, 2008; see Figure 10). Furthermore, multiparous dams (2 litters) consistently responded faster than primiparous subjects.

Similarly, multiparous more effectively hunt crickets as well as traverse a smooth rod when compared with less experienced subjects (Lambert & Kinsley, 2008). These improvements in hunting performance persist after the weaning of young (Kinsley et al., 2014).

More recently Hussain et al. (2013) examined the possible effects of reproductive experience on estrogen-mediated place and response learning. Both nulliparous and primiparous rats that had given birth previously were administered a low or high dose of estradiol and then assessed for their responses on a place and task test. The results indicated that at a low dose of estradiol the primiparous dams were unaffected in the learning tasks, whereas in the nulliparous subjects, estradiol facilitated the learning tasks. Thus, consistent with prior endocrine studies, parity results in a shift in estrogen sensitivity in regulating this aspect of learning and brain functions. Taken together, these results support the concept that prior parity affects a range of behavioral processes that include maternal care, learning, and perhaps hunting strategies which help the experienced mother adapt to environmental demands to facilitate continued reproductive success.

Summary and Future Directions

The experiences of pregnancy and lactation can profoundly remodel a number of biological systems, modifying the female's underlying physiology for what appears to be for the remainder of her life. Shifts in endocrine, neuroendocrine, neurochemical, neuroanatomical, immunological, and behavioral activities transform the female's biological state, resulting in changes in neural, hepatic, mammary, and immune processing. Significant shifts appear to occur within the brain's dopaminergic and neuroendocrine systems that help adapt the mother after single or multiple pregnancies and lactations to cope with her new life state as a mother. Some key questions remain that need to be answered so that a more comprehensive understanding of the biological and behavioral changes that occur are understood. First, whereas it appears that the combination of pregnancy and lactation are necessary to induce many of the identified changes, it is important to clarify the precise roles of pregnancy, lactation and the accompanying behavioral events in these modifications. It is possible that the thresholds for inducing endocrine, neuroendocrine and behavioral changes, for example, may differ. It is apparent that the establishment of maternal memory requires limited maternal care postpartum (Bridges, 1975), whereas a similar experience fails to modify dopaminergic changes in prolactin regulation. That is, experiencing pregnancy with minimal postpartum pup exposure in rats fails to alter the female's responsiveness to haloperidol-induced PRL release later in her life (Byrnes and Bridges, 2005), whereas pregnancy and a full lactational experience does (Bridges et al., 1997). The extent of the reproductive experience as well as multiple experiences in modifying the range of factors altered is not

known, but can be experimentally addressed. Next, the permanency of the reported changes in biological processes remain to be established. Although many of these changes persist well past the cessation of lactation and the weaning of young, it is unknown whether the changes would revert to the initial nulliparous status over sufficient time. Some permanency seems likely based upon the prolonged retention of maternal memory in primiparous mother rats for almost three months (Scanlan et al., 2006). Third, the mechanisms that mediated these changes in neurobiological and biological functions are really only beginning to be understood. Given PRL's role in the induction of maternal behavior (Bridges et al., 1985) and the established increase in neural PRL sensitivity (Anderson et al., 2006) brought about by prior parity, it is interesting to postulate that the rapid expression of maternal memory following reproductive experience may be mediated by increased neural sensitivity to PRL or PRL-like molecules acting at the level of the MPOA (Bridges et al., 1990). Also, might pregnancy-related neurogenesis contribute to behavioral and physiological changes or are other unidentified molecular, epigenetic and/or genetic events driving the reproductive experience-induced plasticity. From a human health perspective, much like in the case of sex differences, considerations of drugs or substances that interact with these remodeled systems should be weighed in the determinations of dosing and treatment timing.

Finally, it is also important to determine the biological meaningfulness of these changes. Do these changes make the female a more effective provider both from a metabolic and behavioral perspective? Given Jay Rosenblatt's superb scientific training with the animal behaviorist and ethologist TC Schneirla, a question expected from Jay would be - *what is the ethological or adaptive significance of these transformational changes produced by motherhood?* This type of question, approach, and thinking will help us to understand the impact of the biological changes associated with motherhood and the longer term implications of such changes across the adult female's lifespan.

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Highlights

- Pregnancy, birth and lactation result in long-term changes in biological processes
- Neural, immune, hepatic and mammary set points shift after reproductive experience
- Reproductive experience induces long-term neuroanatomical and neurochemical changes
- Reproductive experience reprograms hormonal and neural hormone receptor systems
- Reproductive experience enhances maternal and other behavioral responses

Long-Term Effects of Reproductive Experience on a Set of Biological Systems

Figure 1.
Constellation of systems affected by the prior reproductive experiences of pregnancy, parturition and lactation.

Figure 2.

Plasma PRL levels in primigravid (○) and multigravid (●) rats during a 24 hr period starting at 1000 h on day 7 of pregnancy. Numbers on the abscissa refer to the time of day, e.g. 10 is 1000 h. * Significantly different from multigravid animals. Taken with permission from Pergamon Press Ltd, *Life Sciences* 53:439, 1993.

Figure 3.

Effects of reproductive experience on plasma prolactin in ovariectomized post-lactational primiparous (n=13) and nulliparous (n=11) rats after s.c. injections of the dopamine antagonist, haloperidol (HAL). Subjects were treatment with a low dose (0.2 mg/kg) of HAL and 2 days later with a high dose of HAL (1.0 kg/mg). * $P < 0.05$ versus same dose nulliparous subjects. With permission from S. Karger AG, Basel, *Neuroendocrinology* 66:321, 1997.

Figure 4.

The effect of apomorphine (1.0 mg/kg) on the induction of oral stereotypy in age-matched, intact nulliparous (n=9) and non-lactating, multiparous (n=8) female rats tested during diestrus. Results are presented as the percentage of animals within each group that displayed oral stereotypy over 15 minutes. * $P < 0.05$ versus nulliparous subjects. With permission from Elsevier Science Inc., *Pharmacology, Biochemistry and Behavior* 68:481, 2001.

Figure 5.

Percentage of age-matched, primiparous and multiparous (second litter), lactating rats displaying full maternal behavior or retrieval of at least one test pup to the nest starting 30 minutes following bilateral MPOA infusions of β -endorphin (0.29 nmol). A lower percentage of primiparous mothers exhibited retrieval and full maternal care during the 60 minute test session. With permission from Elsevier Science Publishers, *Brain Research* 580:241, 1992.

Figure 6.

Left Panel – mRNA levels for prolactin receptor (long form) in the MPOA, PVN and ARC regions, micro-dissected from cycling virgin (nulliparous) and non-lactating, primiparous rats treated with ovine PRL (250 µg, sc) or vehicle 2 h previously on the day of diestrus. * $P < 0.05$ vs. the appropriate vehicle controls; **, main effect of reproductive experience, $P < 0.001$. With permission from The Endocrine Society, *Endocrinology* 147:4688, 2006.

Right Panel – Effect of reproductive experience on numbers of pSTAT5-immunoreactive cells in response to graded doses of prolactin (2.5, 100 or 4000 ng/rat icv) or vehicle in the MPOA. Within bars: ** $P < 0.001$ vs. corresponding vehicle controls; above bars: * $P < 0.05$ ** $P < 0.001$ between virgin and primiparous rats at the same prolactin dose. With permission from The Endocrine Society, *Endocrinology* 152:1979, 2011.

Figure 7.

Effects of parity and postpartum maternal care on maternal memory in rats. Groups were either allowed to give birth and care for their young for 21 days or 4–6 hours, caesarean sectioned on day 21 of pregnancy and given no postpartum pup contact or were inexperienced adult, virgin rats. All subjects were tested for maternal behavior towards foster young 25 days after last contact with pups or after no prior pup contact (caesarean group and virgin subjects). Interaction with pups following birth for 4–6 hours was sufficient to enhance maternal memory 25 days later. In the absence of pup contact, the latency of caesarean operated dams was similar to virgin subjects and significantly longer than the 21 day and 4–6 hr groups. * $P < 0.05$ versus both caesarean and virgin groups. Adapted with permission from Brain Research Publications, Inc., *Physiology and Behavior* 14:245, 1975; 18:487, 1977.

Figure 8.

Elevated plus maze (EPM) responses of re-induced maternal nulliparous and non-lactating, maternal primiparous rats. Home cage maternal care was re-induced 55 days after last exposure to her own young (primiparous) or foster young (used to induce maternal care in the nulliparous subjects). After home cage maternal behaviors were re-induced, subjects were tested on the EPM for responses under two conditions, either with pups out of reach placed in a container at one end of an open arm or in the absence of pups. The re-induced primiparous subjects spent more time on the open arms and traveled a further distance than did the re-induced nulliparous females when pups were present. Main effect of parity ($P < 0.02$) and pup exposure ($P < 0.01$). * $P < 0.01$ versus designated groups. With permission from the American Psychological Association, *Behavioral Neuroscience* 120:676, 2006.

Figure 9.

Effects of estrous cycle and reproductive experience on EPM responses in young and middle-aged females. * $P < 0.05$ as compared to age-matched, nulliparous controls at the same phase of the estrous cycle. # $P < 0.02$ compared to primiparous, middle-aged females in constant estrus (CE). With permission from Elsevier Inc., *Hormones and Behavior* 50:70–76, 2006.

Figure 10.

Effects of single and repeated births in rats on learning in the dry land maze (DLM) test across the adult lifespan. * $P < 0.05$ and ** $P < 0.001$, significantly different between primiparous and multiparous subjects; # $P < 0.05$ and ## $P < 0.001$, significant differences between primiparous and nulliparous subjects. With permission from Blackwell Publishing Ltd., *Journal of Neuroendocrinology* 20:515, 2008.

Table 1

Reproductive Status of Adult Females

Reproductive Status	Description
Nulliparous	Never Has Given Birth
Primigravid	First or Initial Pregnancy
Primiparous	Gave Birth Once/First Birth
Multigravid	Multiple Pregnancies
Multiparous	Gave Birth Multiple Times (2)