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The brain oxytocin and corticotropin-releasing factor systems in grieving mothers: What we know and what we need to learn.

Short title Neuropeptides after offspring loss

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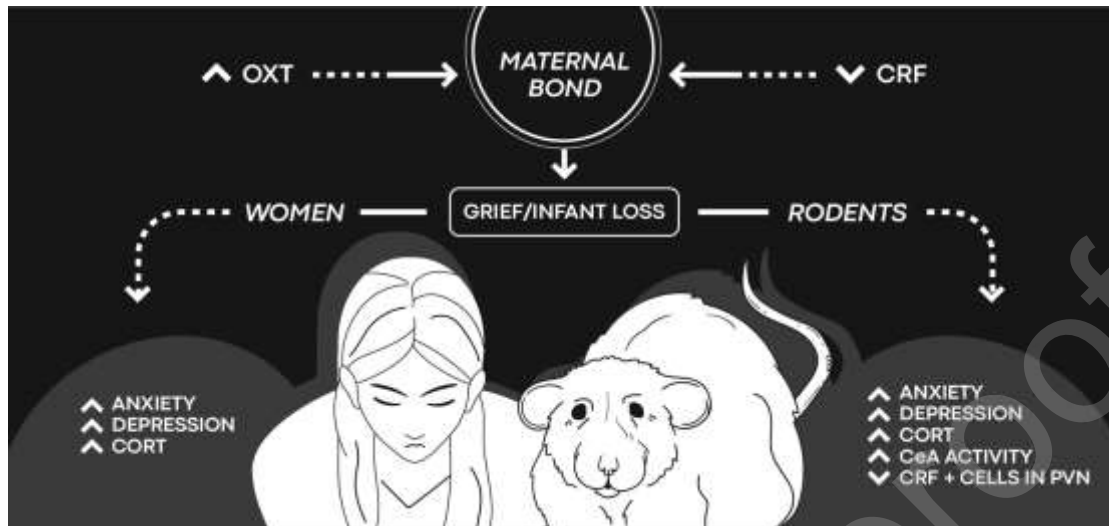
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Graphical abstract

Graphic representation of infant loss-mediated impact on emotionality and neuropeptide systems – what is known in women and rodents. Illustration by Carola Demarchi.



Highlights

- The mother-child bond is the most enduring bond in life
- Losing a child is devastating, increasing the susceptibility for grief disorders
- We provide evidence for potential roles of OXT and CRF systems in grieving mothers
- These neuropeptide systems are involved in maternal behavior and stress response
- Future research is needed to understand the neurobiology of grief in motherhood

Abstract

The bond between a mother and her child is the strongest bond in nature. Consequently, the loss of a child is one of the most stressful and traumatic life events that causes Prolonged Grief Disorder in up to 94% of bereaved parents. While both parents are affected, mothers are of higher risk to develop mental health complications; yet, very little research has been

done to understand the impact of the loss of a child, stillbirth and pregnancy loss on key neurobiological systems. The emotional impact of losing a child, e.g., Prolonged Grief Disorder, is likely accompanied by dysregulations in neural systems important for mental health. Among those are the neuropeptides contributing to attachment and stress processing. In this review, we present evidence for the involvement of the brain oxytocin (OXT) and corticotropin-releasing factor (CRF) systems, which both play a role in maternal behavior and the stress response, in the neurobiology of grief in mothers from a behavioral and molecular point of view. We will draw conclusions from reviewing relevant animal and human studies. However, the paucity of research on the tragic end to an integral bond in a female's life calls for the need and responsibility to conduct further studies on mothers experiencing the loss of a child both in the clinic and in appropriate animal models.

Keywords: CRF; grief; infant loss; grieving mothers; oxytocin; stress

1 | Introduction

Positive and intact social bonds are important in the social life of humans as well as of many other social species [1]; such positive social bonds ensure the individual's psychological and physiological well-being. Consequently, these can be dramatically impacted by the loss of a bond, i.e., due to separation or death. Among the emotional bonds found in nature, the maternal bond is arguably the most important and often the most long lasting in the course of our lives ([2]; [3]). The early mother-child bond not only shapes the future 'me', but has a great impact on the mother as she undergoes a number of peripartum adaptations in order to become and stay maternal [4]. It is undisputed that a dramatic event in the peripartum period like the termination of the mother-child bond due to, e.g., pregnancy loss, stillbirth, crib death, illnesses or accidents, is considered an emotional trauma that devastates the

psychophysical well-being of the mother and family ([5]; [6]; [7]). The loss of a child, consequently, is a dramatic and stressful event that afflicts many families around the world. In fact, globally in 2019 it was estimated that for every 71 live births there was one stillbirth, which is defined as no sign of life at 28 weeks of pregnancy or later [8]. In addition, the global child mortality rate is estimated to be 4% [9]. Statistics on miscarriage are even higher, as this is the most common complication of early pregnancy [10]. Among women who are aware of their pregnancy, the miscarriage rate is 10% to 20% [11] with 45% of pregnancies ending in miscarriage in women over the age of 40 [11]. Therefore, with so many mothers experiencing the loss of a child there is an urgent need to understand the neurobiology of bereavement in motherhood.

Conceptually, grief is an attachment reaction while bereavement is the period in which the person suffers with the loss [12]. Psychological symptoms following the loss of a loved one can range from feeling insecure, agitated, aggressive, anxious and having difficulties accepting the loss to depressive states that can lead in 7% to 10% of the bereaved population to Prolonged Grief Disorder (PGD) ([13]; [14]; [15]; [16]) i.e., when the symptoms last more than 6 months. However, in bereaved mothers and fathers, up to 94% develop PGD ([17]; [18]). Moreover, PGD is frequently comorbid with depression, anxiety and PTSD with up to 75% of individuals with PGD developing symptoms of one or more of these disorders [19]. Furthermore, recent research has shown that women who experience early pregnancy loss (loss before 12 weeks of gestation) have high levels of posttraumatic stress, anxiety and depression that remains clinical significant 9 months later [20]. It is also not surprising that stillbirth is accompanied by high levels of anxiety and depression in the mother for up to 3 years [21]. The neurobiology of grief is an emerging research field that is constantly growing but unfortunately, scientific research has been influenced by a strong sex

bias that has created a lack of knowledge of female physiology [22], thereby also leading to the little we know about grieving mothers' neurophysiology and emotionality. Hence, we aim to review the current literature on the neurobiological impact of losing a child on the mother with focus on neuropeptide systems known to facilitate (oxytocin; OXT) or to hinder (corticotropin-releasing factor; CRF) adequate mothering ([23]; [24]; [25]; [26]) (for a more general review of neuroendocrine mechanisms in grief and bereavement see [27]). Given the severe paucity of scientific research in this field, we decided to include examples of relevant studies on repeated forced mother-offspring separation. This repeated mother-offspring separation paradigm is not the same as permanent removal of the offspring but is a forced and often unpredictable separation that can provide knowledge of the neurophysiological systems at play when a mother-offspring bond is severed. The impact of repeated mother-offspring separation on the mother spans from changes in behavior to molecular levels in experimental animal models and women, and could further help to identify gaps and possible future research directions on neurobehavioral processes in grieving mothers.

1.1| The maternal bond

Among the central evolutionary force in life is the ability to develop specific and even long-lasting bonds ([28]; [29]). In particular, the maternal bond is considered not only the basis to form social bonds, but also the strongest and most enduring bond in life for both participants in the mother-child dyad ([30]; [31]). The experience of becoming a mother involves adaptations on hormonal, neuronal and, consequently, behavioral levels all of which ensure the survival of the offspring ([32]; [33]; [34]; [35]; [2]; [25]; [26]). Maternal behavior is defined as the sum of all behaviors aimed at maintaining the offspring's well-being. After giving birth, the rodent mother is spontaneously attracted to her pups, which is

triggered by numerous sensory stimuli (olfactory, auditory, visual, chemical), ([36]; [37]; [38]).

In rodents, the reproductive experience itself is capable of affecting the maternal brain on multiple levels, including emotionality, stress response and cognition [39]. Curiously, in women the experience of pregnancy causes neuroplastic changes, i.e., reducing gray matter volume in many brain areas associated with social cognition, that are predictive of greater maternal postnatal attachment [40]. In line with this, lactating rodents, with lower levels of neurogenesis in the hippocampus, show increased memory during the postpartum period and even after weaning ([41]; [42]; [43]; [44]; [45]). This suggests a fine-tuning of neural systems with the transition to motherhood to ensure offspring survival. The relationship between the mother and the infant is directly linked to the neurobiological and behavioral development and well-being of both subjects ([46]; [47]). For example, a better quality of maternal care has long-term positive effects on pups, reducing anxiety and increasing sociability ([48]; [30]). Consequently, disrupting the bond, either repeatedly or permanently can have detrimental impact on the offspring, as has been shown over the past decades (for reviews see: [49]; [50]). For example, a reduced quality of maternal care has a negative effect on learning and memory and on the neuroendocrine response to stress in the adult offspring [51]. The reduction in maternal care or repeated separation from the mother in the postnatal period causes severe long-lasting consequences, such as increased anxiety [52], altered stress response [53] and epigenetic changes in the central nervous system of the adult offspring ([54]; [55]). It is unfortunate, though, that only a few studies have characterized the infant-loss mediated impact on maternal neurobiology.

1.2 | Role of the OXT and CRF systems in the healthy maternal brain

The aim of this review is to collect the information on the consequences of breaking the mother-child bond on the maternal brain with respect to the “pro-maternal” OXT and “anti-maternal” CRF neuropeptide systems [25]. As these neuropeptide systems are also involved in the etiology of stress-related disorders in both rodents and humans, and that losing a child is a severely stressful experience that dramatically affects the maternal brain, these neuropeptide systems are promising candidates with respect to the underlying physiology of emotional and behavioral consequences in grieving mothers. Our first step here is to briefly summarize our knowledge of these neuropeptide systems in the maternal brain. See the following for more in-depth reviews on the brain OXT ([2]; [56]) and CRF systems ([57]; [25]).

1.2.1 | The OXT system is activated in lactation and promotes maternal behavior

OXT is a nonapeptide synthesized primarily in two hypothalamic nuclei, i.e., the paraventricular nucleus (PVN) and the supraoptic nucleus (SON). The magnocellular OXTergic neurons project from these two nuclei to the neurohypophysis, where OXT is stored in the axon terminals and then secreted into the blood. The peripheral actions of OXT include the stimulation of the myometrium contractions during labor and secretion of milk during lactation [56]. Concurrently, OXT is also released in the central nervous system in regions such as the PVN, SON, septum, hippocampus and olfactory bulb ([58]; [59]; [60]; [61]; [62]; [63]).

Central OXT acts as a modulatory factor of emotionality, reducing anxiety and the response to stress on one hand and favoring social and emotional bonds, and social behavior on the other hand ([64]; [23]; [65]; [66]; [56]). In this context, OXT is involved in mediating both maternal care and maternal aggression as shown in rodents ([67]; [68]; [69]; [70]; [71]; [72]; for in-depth reviews see [2]; [73]; [56]). Therefore, it is not surprising that both central and

peripheral OXT levels increase during the postpartum period as demonstrated in rat and sheep mothers ([59]; [74]; [75]; [64]). Additionally, OXT receptor (OXT-R) density increases around parturition in several brain areas known to be involved in maternal behavior, including the medial preoptic area (mPOA) and bed nucleus stria terminalis ([76]; [77]). In parturient rats, central administration of an OXT-R antagonist blocks the onset of maternal care [78]. In lactating rats, intracerebroventricular (icv) infusion of OXT does not further increase maternal care [79], while an OXT-R antagonist lowers the level of pup-directed behavior ([70]; [24]). In support, mice with a knockout for the OXT-R show impaired maternal care [80]. In women, high levels of plasma OXT are associated with high levels of maternal attachment ([81]; [82]). Conversely, low plasma OXT levels are thought to be a sign for an increased risk for postpartum depression ([83]; [84]; [85]). However, it is important to underline that the release of OXT into the peripheral blood stream can be independent of central release and, thus, does not necessarily reflect brain OXT levels [86]. Recent genetic studies in humans reveal that the quality of mothering is influenced by single nucleotide polymorphisms in the genes for OXT (e.g., rs2740210; rs4813627; [87]) or OXT-R (e.g., rs968389; [88]).

1.2.2 | The CRF system activity is dampened in lactation and impairs maternal behavior

The CRF system consists of four polypeptidergic ligands (CRF, Urocortin 1-3), CRF binding protein (binds free CRF and Urocortin 1 thereby reducing their availability for the receptors), and two receptors (CRF-R1 and CRF-R2). These members of the CRF system are expressed widely throughout the brain [57]. One of the main roles of CRF is driving the stress response via the hypothalamo-pituitary-adrenal (HPA) axis. The response to stress is triggered in the parvocellular part of the PVN leading to CRF release into the portal blood. Here, it elicits the

release of ACTH from the posterior pituitary gland into the main blood stream. In the adrenal cortex, ACTH stimulates the release of cortisol (humans) or corticosterone (rats, mice), which in turn exerts a negative feedback on various levels including PVN, hippocampus, and pituitary gland. In the brain, increased CRF system activity is affecting numerous behavioral outcomes including impairment of maternal behavior. Therefore, it is not surprising that one of the peripartum adaptations is a dampening of the CRF system activity (for a comprehensive review see [25]).

The HPA axis activity is significantly reduced in lactating rats, mice and humans ([89]; [90]; [91]); the brain of lactating rats, in general, is less responsive to CRF compared to virgin females ([92]; [93]). This includes, e. g., decreased CRF mRNA in the central amygdala (CeA) [94], and the parvocellular PVN ([89]; [94]; [95]; [96]). Consequently, a centrally activated CRF system impairs maternal behavior in lactating mouse and rat mothers ([95]; [97]; [98]), and can even induce pup-killing in ovariectomized female rats [99]. In addition, the involvement of both CRF-R subtypes can differ. In the antero-dorsal BNST, e.g., CRF-R1 activation impairs maternal behavior to a greater extent compared to CRF-R2 activation [97], whereas opposite effects are observed in the medial-posterior BNST ([100]; [101]).

Therefore, those and other studies (see review by [25]) demonstrate that a downregulation of the CRF system peripartum is a prerequisite for mothers to exhibit adequate maternal behavior. In support, an upregulated CRF system is thought to be a risk factor for psychological disorders ([102]; [103]; [104]). Hence, any maladaptation of the CRF system in general - and in the peripartum period in particular - likely results in serious consequences like increased risk to develop emotional disorders, which affect at least 15% of mothers [105]. Importantly, CRF is one of the factors known to contribute to such maladaptation ([106]; [107]).

2| Grief and bereavement of the offspring

Worldwide, approximately 2.6 million newborns die each year [108]. Infant loss is a tragic life-changing event for parents which often causes PGD, including symptoms of depression and anxiety ([109]; [110]; [111]), e.g., the bereaved parents show higher levels of emotional distress and post-traumatic symptoms when compared to non-bereaved parents ([108]; [112]). In fact, the loss of one's child is considered the most vulnerable type of bereavement ([113]; [114]; [115]). It can even cause physical pain, i.e. significantly increasing pain intensity, compared to the loss of a partner or parent [116]. It is not surprising that the first six months following the death of a child are the most intense months of psychological stress for mothers [117]. The extent to which mothers suffer from the consequences of child loss can decline over time ([118]; [14]). A study reports that one month after their child dies, 17% of parents (both the mother and father) suffer from post-traumatic stress disorder, which decreases to 9% in the following 3-12 months [119]. While both parents are affected by the loss of the child, mothers are of higher risk to develop mental health complications ([120]; [121]; [122]) with the majority suffering from chronic depression [113]. Surprisingly, the form of grief following the loss of a child is still underestimated and not well understood ([5]; [123]; [124]). Studies on grieving mothers find no differences in the symptomatology across the various types of infant loss (e.g., prenatal versus postnatal) [125].

3| The CRF system after offspring loss in rodents

The HPA axis underlies peripartum adaptations; its activity is different compared to virgin female or male animals due to the role of components of the HPA axis in offspring care [126, 127]. Under normal, non-stress conditions, lactating mothers show hypercortisolism,

which is required to meet the constantly high energy demand in lactation ([128]; [129]; [130], [96]) as well as form maternal memory [127]. Under acute stress conditions, the HPA axis reactivity is altered to enable the mother to react adequately ([128]; [131]; [132]; [133]; [96]; [94]; [134]). In the case of breaking the maternal-offspring bond, it is likely that this acts as a chronic stressor, or abnormal event, thereby altering the function of the maternal HPA axis on many levels and for a longer period time ([135]; [105]; [136]). In support of this notion when looking at the studies that remove the litter completely from the mother shortly after birth, more analogous to the clinical situation of infant loss, it has been shown that these mother rats have increased depressive-like behavior, increased anxiety and an impairment in memory weeks later ([137]; [138]; [44]). These behavioral effects weeks after offspring removal occurred despite the fact that removal of the litter shortly after birth resulted in serum levels of corticosterone returning to virgin levels 5 days later [139]. Unfortunately, we lack detailed knowledge on the effects of infant or child loss on the maternal brain in rodent literature and how it may differ from both the maternal and virgin states. This is despite the fact that a large body of research has analyzed the consequences of mother-pup separation on neurobehavioral outcomes in offspring, perhaps most popularized with the research of Harry Harlow. Of the few papers that have investigated the effects of mother-offspring separation on the mother, the majority of studies have adopted the maternal separation paradigm with slightly varying timelines and durations of separation.

Given that there are no studies of offspring loss that thoroughly investigate the neuroendocrine effects in the mother, we extend the discussion here to studies that investigated the OXT and CRF systems by using repeated mother-offspring separation paradigms. Such paradigms which consist of forced separation between mother and

offspring are generally applied during the first postnatal days, typically during the first 2-3 weeks after giving birth, either for a short (15-30 minutes per day) or a prolonged period (3-6 hours per day). The studies that adopt this paradigm demonstrate an impact of the separation on both behavioral and molecular levels in the mother (Table 1). For instance, maternal care is influenced by the duration of separation from the pups. Repeated short separation induces an increase in licking and grooming and arched-back nursing ([140]), whereas repeated prolonged separation leads to reduced maternal care ([141]; [142]). However, a recent study that applied daily 4 hour-separations from postnatal days 2-20 reports increased licking and arched-back nursing behaviors in rat dams [143]. In addition, the long-term separation can also impair the mothers' short-term memory [144]. With respect to emotionality, repeated prolonged separation between 3 and 6 hours for 2-3 weeks postpartum results in increased depressive-like behavior [145] and an anxious phenotype in the mothers ([146], [144], [140], [147]; but also see [143]). The latter is speculated to be due to an increased cellular activity of the CeA [144].

From a molecular point of view, the literature on neuroendocrine effects of mother-offspring separation on the maternal brain is limited and mostly focuses on glucocorticoids. Following repeated short separation, maternal plasma corticosterone levels increase [146]. Curiously, in another study, complete removal of the offspring from the postpartum dams for 3 days decreased corticosterone to levels observed in virgin rats [148]. Repeated prolonged separation during the first week postpartum increases glucocorticoid receptor gene (Nr3C1) expression in the mothers' hippocampus ([140]; but also see [143]). Previous studies show that variations in the expression pattern of this gene in the hippocampus could induce a vulnerability to potential injuries following chronic stress [149]. Furthermore, the expression of Morc1 mRNA, which is connected to chronic stress experience and depression

([150]; [151]; [152]), is increased in the medial prefrontal cortex (PFC) of mothers after repeated prolonged pup-separation [143]. Repeated prolonged separation decreases the density of positive CRF cells in the PVN of the mother when compared to mothers exposed to repeated short separation [153]. These data demonstrate a strong impact of pup separation on the maternal stress axis and emotionality which may have life-long effects on the well-being of the mother. More concentrated research efforts are needed on the neurophysiology of grieving mothers in order to identify potential treatment targets to regulate the HPA system and ease the impact of the loss.

4| The OXT system after offspring loss in rodents

The OXT system plays a fundamental role in maternal behavior and at the same time in regulating the stress response ([2]; [56]). During pregnancy and lactation, OXT system activity is elevated ([2]; [154]); e.g., the expression of, and binding to, OXT-R as well as the number of OXT positive cells in hypothalamic regions is increased in non-stressed mothers ([155]; [156]). Chronic central OXT infusion attenuates the neuronal and neuroendocrine responses to stress in virgin female rats [157] further supporting the involvement of endogenous OXT in the attenuated stress responses during the peripartum period. Importantly, the stress system is also capable of manipulating the OXT system as demonstrated in an animal model of partner loss in the monogamous, biparental prairie vole (*Microtus ochrogaster*) [158], which have evolved as an important animal model to study social bonds (for detailed reviews on their social system and studies on loss see [159]; [160]; [161]; [3]). Briefly, losing the female partner, i.e. permanent separation, acts as a chronic stressor on the male [162], thereby causing increased CRF-R2 activation, which suppresses OXT mRNA expression in the PVN and reduces local OXT signaling in the nucleus accumbens

(NAcc) shell. The result is an adverse emotional state that shares common mechanisms with bereavement ([3]; [1]). Importantly, this state can be reversed by chronic administration of OXT or deactivation of CRF-R2 in the NAcc shell [158]. Interestingly, the OXT and CRF systems might interact directly via neuronal coexpression of OXT-R and CRF-R [163]. For example, in the NAcc CRF positive parvocellular PVN neurons express OXT but not OXT-R, while magnocellular PVN neurons coexpress CRF, CRF-R2, OXT and OXT-R, allowing these positive CRF neurons to respond to OXT release and *vice versa* ([164]; [163]). Similarly, separation from the male partner induces chronic stress in the lactating female prairie vole, increasing the anxious phenotype and passive stress-coping [165]. In socially separated female prairie voles, i.e. being isolated from a female sibling for 4 weeks, repeated subcutaneous administration of OXT buffered the separation-induced increase in depressive-like behavior and cardiac dysfunction [166]. However, the OXT systems' role in female prairie vole mothers following separation remains to be investigated.

With respect to mother-offspring bond disruption, most studies use the paradigm of repeated short or long maternal separation, as mentioned in section 3, to analyze potential dysregulations of the OXT system (Table 1). For example, repeated short separation from postnatal days 1 to 22 increases OXT-R expression in numerous brain regions involved in the response to stress and/or maternal behavior, including the hippocampus, CeA, PFC, mPOA, lateral septum, and NAcc shell compared to a control group [167]. Furthermore, repeated short separation from pups increases the number of OXT-immunoreactive cells in the PVN whereas repeated prolonged stress reduces it in the caudal PVN compared to non-lactating rats [153]. This is an interesting finding as it suggests that prolonged maternal separation stress is able to reverse the peripartum adaptation-associated increase in OXT-immunoreactive cells [153]. Different to those effects on the OXT system in the brain,

plasma OXT levels are similar in rat mothers exposed to repeated short or prolonged separation from offspring [146]. Moreover, even total deprivation of pups for 20 hours over 4 consecutive days does not alter plasma OXT levels compared to control in rat mothers [168]. However, it is important to keep in mind that removal of pups from the mother also results in the absence of the milk ejection reflex and, consequently, an absence of stimulation of the mammary glands, which can stop lactation. Therefore, it is not surprising that the same study reports a cessation of lactation in the long-term separated group [168]. The cause of this cessation of lactation is the abnormal electrical activity of OXT neurons, which do not respond adequately when not being stimulated regularly by suckling [168]. Another factor that needs to be considered when studying the maternal brain is the age of the pups or rather the progress of lactation. In the biparental, monogamous prairie voles, removal of the pups for 30 minutes strongly activates OXT neurons in the PVN on postnatal days 2 and 9, whereas there is only minimal activity on postnatal day 21 suggesting that the functional plasticity of the OXT system is associated with parental behavioral and the growth of the pups [169].

5| The CRF systems after infant loss in humans

Studies in humans evaluating the endocrine effects following bereavement use blood cortisol levels as a primary indicator of stress or rather of chronic stress (Table 2). Cortisol is released from the adrenal gland, and activation of the HPA axis increases circulating cortisol levels. Moreover, high cortisol concentrations can cause a dysregulation of the HPA axis, a process that has been related to a state of chronic stress as seen, e.g., in major depressive disorders [170]. The first studies that have analyzed the relationship between psychological stress and adrenal gland activity date to more than 50 years ago. In 1964, Wolff and

colleagues analyzed plasma levels of 17-hydroxycorticosteroid (17-OHCS) in parents of fatally ill children, affected by leukemia or other fatal childhood neoplasms. This study demonstrates that the more effectively a parent “fights” against the threat of loss, i.e. the more that control is exercised over feelings, the lower their chronic 17-OHCS excretion rate is [171]. A few years later, a study indicated that the 17-OHCS excretion levels during a pre-child loss period can be significantly different from the bereavement period and that the direction of the difference is an important characteristic of the individual subject [172]. An evaluation of pairs of parents who experienced sudden child death demonstrates no difference in cortisol levels compared with non-bereaved subjects, whereas the bereaved parents score higher on the Beck Depression Inventory test and have increased helper T cells as an indicator of an immunological response [173].

Due to a lack of further data on grieving mothers, we also must consider studies on women suffering from the loss of close persons. In the literature, most studies have analyzed women and men in mourning as one group, without considering potential gender differences or even distinguishing the type of bereavement, i.e., whether a child, a partner, or a family member has deceased. In 2009, Buckley and colleagues analyzed the plasma cortisol level in non-bereaved versus bereaved men and women 2 weeks and 6 months after the loss of a loved one (a partner or a child). Comparing the two time points, the morning cortisol level increased in the bereaved compared to the non-bereaved participants, regardless of gender, suggesting an evident reaction of the HPA axis to the stress of loss. Furthermore, elevated depressive symptoms were found in the bereavement group at both time points [174].

Curiously, a study found that men show lower cortisol levels while women show higher levels at 6 months post bereavement [175]. In a small sample of widows, within the first 6 months of partner loss plasma cortisol levels were significantly higher compared to a sample

of women who were still married [176]. In support of long-term effects, daytime cortisol levels were increased in men even many years after the loss of a loved one [177].

Furthermore, morning and daytime cortisol levels were higher in participants with PGD than in normal grieving or non-grieving participants, the latter two being no different from each other [178]. In a recent review, it emerged that the loss of a loved one can be associated with neuroendocrine alterations both in the early / acute stage and in the late / chronic stage of bereavement [27]. In general, people with PGD have flattened daytime cortisol curves and reduced morning cortisol levels when compared to people in normal bereavement. Furthermore, the closer the relationship to the deceased person was, the more the neuroendocrine system is dysregulated.

6| The OXT systems after infant loss in humans

Similar to rodents, the OXT system facilitates maternal behavior and the maternal bond in humans [82]. Research on the maternal bond identified a potential link between OXT and dopamine in the medial amygdala network, i.e., NAcc, amygdala, and PFC, assuming that both neurotransmitters/modulators contribute to the attachment [179]. Recently, peripartum salivary OXT levels in mothers were found to be moderately associated with maternity blues and significantly correlated with postpartum fatigue [180]. However, only a few studies tried to investigate a potential link between the parental OXT system and separation from the children as a stressor (Table 2). In a study on the effects of intermittent separation, e.g. 15 minutes, and reunion with one's infant, near-infrared spectrometry demonstrated a positive correlation between parental activation of the PFC, parenting and OXT levels [181]. This finding is in line with previous research demonstrating a connectivity to the medial PFC and orbitofrontal cortex from the limbic system, such as amygdala, NAcc,

and hypothalamus ([182]; [183]), thereby suggesting that OXT activates the limbic system directly, and the PFC indirectly, via neural connections. In addition, a study by Rutherford and colleagues using event-related potentials (ERPs) showed that OXT administration can enhance the ERPs elicited by infant distress as compared to infant neutral faces in non-parent women giving more support to the OXT system's role in the modulation of attentional processing of infant stimuli [184].

With respect to grief, studies that have analyzed the association with OXT usually do not distinguish between men and women, nor the type of grief (see section 5). We do know that up to 94% of parents develop PGD after the death of a child ([17], [18]), whereas mourning the death of a loved one causes PGD in 10% of individuals [122]. Women are, in general, more vulnerable to developing PGD [16]. This gender difference is an important factor to consider when investigating OXT and PGD as OXT levels vary with gender [185]. In individuals with PGD, plasma OXT is often measured due to its role as a 'social' neuropeptide and its role in social bonds. Although it is debatable whether plasma OXT reflects brain OXT levels, the direct correlation between peripheral and central OXT levels appears to be the prerogative of non-human animal studies, while in humans a direct correlation is apparent in high stress situations [186]. Indeed, the latter might be the case in PGD, and numerous studies have reported a link between plasma OXT and human behavior (for review see: [187]). In a recent study, Bui and colleagues investigated plasma OXT levels in men and women with a primary PGD and they found that in participants with PGD plasma OXT levels were higher compared to participants with depression, but not different from participants with normal grief experiences. Secondary analyses revealed that the diagnosis of PGD is positively associated with plasma OXT levels in men and women [188]. Hence, it is speculated that the increased plasma OXT levels is reflecting the stress arising from the loss

of a social bond, which is specific to PGD compared to depression [189]. An additional study provides further support for a potential role of OXT signaling in the pathophysiology of PGD by reporting a gene x environment effect between a genetic variant of the OXT-R (single nucleotide polymorphism rs2254298) and greater PGD symptom severity [190].

7 | Summary and conclusions

This review proposes that the OXT and CRF systems are involved in the emotional impact of bereavement and grief in mothers. Rodent mothers that have experienced prolonged separation from their pups show an anxious and depressed phenotype accompanied by impaired short-term memory and reduced maternal care ([137]; [44]; [138]; [144]; [142]; [141]). These phenotypic effects can be partly explained by the involvement of the OXT and CRF systems; alterations in the levels of corticosterone and mRNA of the Nrc1 and Morc genes, respectively, in the hippocampus and in the PFC and a decrease in CRF positive cells in the PVN ([146]; [148]; [140]; [143]; [153]). Repeated short separation from pups induces an increase in OXT-R in numerous brain areas and an increase in OXT positive cells in the PVN while prolonged separation from pups decreases the density of OXT positive cells in the caudal PVN ([167]; [153]). There do not seem to be any alterations in plasma OXT following the separation of the pups while the activity of OXT positive neurons increase following the repeated short separation ([146]; [168]; [169]).

Human studies also suggest the involvement of the two presented neuropeptide systems during grief and loss of a child. In particular, there is a direct correlation between 17-OHCS and cortisol levels with the severity of PGD symptoms ([171]; [172]; [177]; [178]). Bereaved parents have an increased susceptibility to develop depression ([173]; [174]) and grieving women show higher cortisol levels than grieving men [175].

Many studies support the role of OXT in increased emotionality and grief ([82]; [179]; [181]; [184]). Salivary OXT levels are related to maternity blues and postpartum fatigue ([180]). At the molecular level, people with PGD have higher plasma OXT levels than depressed people, and the level of OXT is directly correlated with the severity of PGD symptoms; a severity that has also been shown to be genetically influenced by a single nucleotide polymorphism in the OXT-R gene ([188]; [190]).

The review of the literature leads us to hypothesize that the OXT and CRF systems - and with the latter also glucocorticoids - are involved in bereavement and, more importantly, that further studies are desperately needed. For example, the lack of an adequate animal model for studying the emotional impact following the bereavement of a child is a limiting factor. Studies in such an animal model would open the door to a better understanding of the neurobiology of grief, helping to develop possible precision therapies for people suffering from PGD.

Human studies often do not differentiate between different types of bereavement when studying PGD patients and very few have investigated neurophysiological correlates of bereavement. Thus, future studies on humans should take these factors into account, e.g., conducting surveys among mothers who have lost their child and assessing cortisol and OXT levels in these women, thereby potentially distinguishing the types of loss, from pregnancy to after childbirth and the biomarkers they impact.

8| Outlook

Given the high number of child deaths, stillbirths and miscarriages every year and the resulting psychological impact especially in mothers, there is a need for better in-sight in the occurring dysregulations in the brain of grieving mothers. While psychotherapeutic

interventions for PGD are already deployed ([191]; [192]) they are not specific to child loss. In addition, PGD-specific pharmacological treatments are not available. Thus, rodent research specific to offspring loss is needed to further understand the neurophysiological profiles mediating PGD symptoms such as anxiety and depression. We therefore suggest that future studies of infant loss and maternal grief use rodent models where complete offspring separation occurs. Furthermore, we believe that the use of rodent species such as rats and prairie voles are an excellent starting point for understanding the neurophysiological mechanisms contributing to parental grief given that they are, respectively, the foundational animal models for the study of the neurobiology of maternal care-giving and bonding. In addition to this, and from what has emerged in this review, it is important that both the OXT and CRF systems are studied in these models, and clinical populations, as both systems are candidates in the emotional modulation of bereavement. Thus, treatments targeting the OXT and/or the CRF system could be promising for future research in the context of PGD and PGD after the loss of a child. Much more research is needed in clinical populations and in appropriate animal models to decipher the neuropeptide activities in relevant brain regions and determine how the CRF and OXT systems may be targeted for interventions to alleviate PGD when the mother-child bond is broken.

Declaration of interest

None

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Table 1: Overview of studies related to the OXT and / or CRF systems in rodents after pup loss/separation

Abbreviations: BMS = brief maternal separation; CBG = corticosteroid binding globulin; CeA = central amygdala; Cort = corticosterone; CTRL = control; EPM = elevated plus maze; FST = forced swim test; Hip = hippocampus; IHC = immunohistochemistry; IR = immune-reactive; LG = licking and grooming; LHb = lateral habenula; LMS = long maternal separation; mPOA = medial preoptic area; MS = maternal separation; NAcc = nucleus accumbens; OF = open field test; OXT = oxytocin; OXTR = oxytocin receptor; PFC = prefrontal cortex; PND = postnatal day; SL= lateral septum; SUM = supramammillary nucleus; VMH = ventro-medial hypothalamus.

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MS DURATION	MS PERIOD	BEHAVIORAL TEST	BIOMARKERS	MAIN FINDINGS	STRAIN	REF
Permanent separation within 24 h after parturition	Dams tested 34 or 55 days after parturition	Radial arm maze		Litter removal induced memory impairment and decreased learning strategies	Sprague-Dawley rats	[44]
Permanent separation within 24 h after parturition	Dams tested 30 days after parturition	FST, OF, EPM		Litter removal induced depressive-like behavior without effect on anxiety	Sprague-Dawley rats	[138]
Permanent separation within 24 h after parturition	Dams PND1, PND5, PND14, PND21, PND35		Cort, CBG	Litter removal reduced Cort levels and increased CBG levels by PD5	Sprague-Dawley rats	[139]
Permanent separation for 60 min, 1 day, or 8 days	Dams PND10-PND14 to PND 18-PND22		Cort	Litter removal reduced Cort levels as the pup separation duration increased	Sprague-Dawley rats	[193]
3 days of total separation	PND0 to PND3		Cell proliferation, Cort	Total litter removal reduced Cort levels compared to CTRL group	Sprague-Dawley rats	[148]
20 h / day repeated separation	PND1 to PND4	Maternal care	ACTH, Cort, OXT	LMS induced depression and hypogalactia and reduced OXT activity.	Sprague-Dawley rats	[168]

				Intranasal OXT infusion rescued partially the impaired behavior		
180 min / day repeated separation	PND 2 to PND 7	Maternal behavior, OF, Light/Dark box, social interaction	Nr3c1 expression in Hip	LMS induced anxiety without effect on social behavior; LMS increased Nr3c1 mRNA in the Hip; LMS impaired maternal care	BALB/c mice	[140]
270 min / day repeated separation	PND 1 to PND 21	FST, maternal care, EPM, step down inhibitory avoidance task	C-Fos expression	LMS reduced maternal care, increased anxiety, no effect on depressive-like behavior and impaired memory LMS increased C-Fos+ cells in CeA	Wistar rats	[144]
240 min / day repeated separation	PND 2 to PND 20	Maternal behavior, EPM, marble burying	GABA and Glutamate in serum, Morc1 and Nr3c1 mRNA	LMS increased LG behavior and arched-back nursing; no effect on anxiety;	Sprague-Dawley rats	[143]

Table 2: Overview of studies related to the OXT and / or CRF systems in grieving humans

				LMS increased Morc1 mRNA in the Hip and increased the GABA serum levels		
240 min separation	PND 7 or 8	EPM	C-Fos expression	Litter removal increased anxiety and increased C-Fos+ cells in dorsal / ventral mPOA, LHb, SUM, VMH	Long-Evans rats	[137]
BMS (15 min/day)	PND1 to PND22	Maternal care, EPM	OXT+ cells, OXTR binding	BMS increased maternal care and reduced anxiety BMS increased OXTR+ cells in PFC, Hip, SL, mPOA, NAcc shell, CeA BMS increased OXTR binding in Hip, SL, mPOA, NAcc shell	Wistar rats	[167]
BMS (15 min / day) LMS (360 min / day)	PND 1 to PND 21	Maternal care, FST	OXT and CRF IR cells in PVN	LMS increased maternal care compared to BMS LMS and BMS both increased	Long-Evans rats	[153]

				anxiety in the dams BMS increased OXT-IR cells in PVN compared to LMS and CTRL groups		
BMS (15 min / day) LMS (240 min / day)	During 8 random days in the first 2 weeks postpartum	Spontaneous motor activity, defensive withdrawal test	Adrenal gland weight, ACTH, Cort, OXT	Only BMS induced anxiety Only BMS reduced adrenal glands weight Only BMS increased Cort levels No changes in OXT between groups	Wistar rats	[146]
BMS (15 min / day) LMS (180 min / day)	PND 2 to PND 14	FST, sucrose preference test, EPM	Cort	LMS induced anxiety and depressive-like behavior LMS increased Cort levels	Sprague-Dawley rats	[147]
BMS (15 min / day) LMS (180 min / day)	PND 3 to PND 14	FST, maternal care		LMS induced depressive-like behavior compared to BMS and CTRL groups	Wistar rats	[145]

Abbreviations: CG = complicated grief; MDD = major depressive disorder; OXT = oxytocin; OXTR = oxytocin receptor; 17-OHCS= 17-hydroxycorticosteroid.

GRIEF TYPE	PERIOD	SUBJECTS	METHODS	BIOMARKE RS	MAIN FINDINGS	REF
Child loss	During 2 years after loss	N = 40 parents	Interviews, urine samples	17-OHCS	The adrenocortical excretion levels of an individual anticipating the death of their child is characteristic of that individual only in that situation and is not characteristic of them throughout their life or in another stressful situation	[172]
Sudden death of the child	At 2, 4, 6 and 8 months after loss	N = 11 parents	Blood samples, Back Depression Inventory Questionnaire	Cortisol, T-suppressor cells	Depression in bereaved parents; decreased T-suppressor cells and increased T-helper cells	[173]
Terminally ill children	During child illness	N = 31 parents	Interviews, urine samples	17-OHCS	17-OHCS excretion rates are related to the individual's response to threat	[171]
Bereaved spouses or partners of the deceased and bereaved parents	Within 2 weeks and 6 months after loss	N = 62 bereaved individuals , N = 50 non-bereaved	Questionnaire	Cortisol, lipids	Acutely bereaved individuals had depression, anxiety and anger, as well as higher	[174]

					cortisol, reduced appetite, lower total cholesterol and lower sleep duration	
Partner, child, parent, sibling, friend	At 2 and 5 years after loss	N= 1922 person with no grief; N= 131	Saliva samples, interview, Dutch version of the Inventory of Complicated Grief	Cortisol	2 years after loss, the CG group had lower levels of morning cortisol and lower diurnal cortisol compared to the grief and no-grief groups	[178]
Grandparent, parent, sibling, friend, relative, child	Average loss occurred 9.73 years back	N= 93 bereaved individuals	Questionnaire, Inventory of Complicated Grief, Hamilton Depression Rating Scale	OXTR rs2254298 variant	OXTR genotype is related to CG severity	[190]
Bereaved spouses	At 6, 18 and 48 months after loss	N = 263 bereaved individuals ; N = 293 non-bereaved	Interviews, blood and urine samples	Cortisol	Gender-specific effect: bereaved women had higher cortisol levels than bereaved men at 6 months post-bereavement	[175]
Loss or separation during childhood	(1) Loss of husband < 6 months	N= 9 women (1);	Blood samples	Cortisol, natural killer cells	Bereaved women had increased cortisol levels and	[176]

	(2) husband terminally ill (3) healthy husband	N= 11 women (2); N= 3 women (3)			natural killer cell activity	
Parent, spouse	At least 6 months after loss	N= 47 adults with CG; N= 46 bereaved adults with MDD; N= 46 mentally healthy adults bereaved controls	Structured Clinical Interview, Inventory of Complicated Grief, blood samples	OXT plasma level	CG group had higher plasma OXT compared to MDD group	[188]
Parent loss	Loss or separation during childhood	N= 9 men experienced parent loss; N= 10 men experienced parent separation ; N= 38 men control group	Saliva samples	Cortisol	Higher cortisol in men who experienced parental death during childhood	[177]

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