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ORIGINAL ARTICLE

When mothers neglect their offspring: an activated CRF system in the BNST is detrimental for maternal behavior

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Abstract

Becoming a mother is an intense experience that not only changes a woman's life but is also paralleled by multiple central adaptations. These changes evolve before parturition and continue to persist into lactation, thereby ensuring the full commitment of the mother to care for the newborns. Most of our knowledge on these adaptations that drive the peripartum brain come from rodent animal models. On one side, it is known that maternal behavior is initiated and maternal mood is stabilized by an upregulation of the pro-maternal neuropeptide systems' activity of oxytocin and arginine-vasopressin. On the other side, signaling of the rather anti-maternal corticotropin-releasing factor system triggers maternal neglect and increases maternal anxiety. Here, we discuss how the corticotropin-releasing factor system based in the limbic bed nucleus of the stria terminalis negatively affects maternal behavior and maternal mood. Moreover, we apply microdialysis and acute pharmacological interventions to demonstrate how the corticotropin-releasing factor system potentially interacts with the pro-maternal oxytocin system in the posterior bed nucleus of the stria terminalis to trigger certain aspects of maternal behavior.

Keywords Anxiety · Bed nucleus of the stria terminalis · Corticotropin-releasing factor · Maternal neglect · Oxytocin

Introduction

The incredible experience of becoming a mother is life changing. To be prepared for being the caregiver of a dependent newborn, the expecting mother must go through various adaptations that continue into lactation. Most of our knowledge on those adaptations derives from studies in rodent animal models. Here, maternal behavior is initiated before delivery where the expecting mother starts to show the drive to build a nest. During parturition, the mother cuts through the umbilical cord, devours the placenta, and licks the pups to remove blood and amniotic fluid. Pup-licking and -grooming (L/G) behavior supports the pups to urinate/defecate and persists until weaning. Additionally, L/G strongly supports the healthy psychological development of the young (Champagne and Meaney 2001). Furthermore, a valuable parameter for maternal care quality is illustrated in the dam's various nursing positions. Nursing can occur in a blanket posture, a crouching position, or the arched-back nursing (ABN) posture, which is a quiescent high-crouch nursing position (Klampfl et al. 2014, 2016a; Stern and Johnson 1990). Furthermore, the mother retrieves scattered pups to keep them safe within the nest. In this context, defending the pups against a potential threat, such as hostile conspecifics or even predators, is another important aspect of maternal behavior termed maternal aggression (Bosch 2013; Lonstein and Gammie 2002). In order to adequately protect the pups, the innate anxiety of the lactating mother needs to be reduced (Bosch 2011; Bosch 2013), which is part of the peripartum adaptations (Lonstein 2007).

All these adaptations are mediated by various changes throughout the maternal brain. Two main facilitators of maternal behavior are the brain neuropeptides oxytocin (OT) and arginine-vasopressin (AVP), in conjunction with their receptors (for review see (Bosch and Neumann 2012; Jurek and Neumann 2018)). During lactation, the expression of these receptors is increased and/or the release of OT and AVP is elevated in response to pup stimuli depending on the brain region. Key brain regions for maternal behavior are the medial

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preoptic area (MPOA) and the bed nucleus of the stria terminalis (BNST). Numan and Insel (2003) even refer to them as a "super-region" for maternal behavior as they lie adjacent to each other and are neuronally interconnected (Numan and Insel 2003).

Role of the BNST in the maternal brain

The BNST is a complex brain region within the limbic system consisting of several cyto- and chemoarchitecturally distinct subnuclei, which receive projections from the central and medial amygdala, the medial prefrontal cortex and the periaqueductal gray (PAG), among others (Herman et al. 2003; Radley et al. 2009; Spencer et al. 2005). The BNST can roughly be divided into the anterior (aBNST) and posterior division (pBNST). The anterior part is mainly connected with hypothalamic and brainstem regions associated with autonomic activity (Dong et al. 2001a), while the posterior division is involved in controlling neuroendocrine systems and social behaviors (Dong et al. 2001a; Dong and Swanson 2004). For example, ascending projections from the PAG to the aBNST/MPOA are thought to play an important role for engaging in ABN as they inhibit other active behaviors. In return, the PAG receives inhibiting projections from the aBNST/MPOA, thereby stimulating active maternal behaviors, such as L/G or retrieving the pups (Numan and Insel 2003). Interestingly, the BNST and the MPOA mediate maternal care and/or maternal aggression via OT and AVP signaling (Bosch and Neumann 2012). In the MPOA, reduced signaling of OT (by means of OT receptor antagonist) or of AVP (by means of V1a receptor antagonist or antisense oligodeoxynucleotide) delays the onset (Pedersen et al. 1994) and ongoing of maternal care (Bosch and Neumann 2008; Bosch and Neumann 2012). In addition, MPOA-released AVP mediates pup retrieval; signaling of V1a receptors promotes this behavior, whereas V1b receptor gating prevents it (Bayerl et al. 2016). In addition, V1a, but not V1b, receptor binding is upregulated postpartum compared to the virgin brain, thereby enabling pup retrieval in the lactating mother but preventing it in virgins (Bayerl et al. 2016). Hence, well-adapted activation patterns of both receptor subtypes in the MPOA are necessary to ensure maternal motivation. Within the posterior BNST, blocking V1a receptors decreases maternal aggression without affecting maternal care. Additionally, local infusion of OT into the anterior BNST does not alter pup retrieval (Consiglio et al. 2005). This suggests that the OT system in the BNST, at least in the anterior portion, plays a minor role for maternal behavior in general. However, in this review, we show new data demonstrating an impact of OT activity in a different part of the BNST on maternal behavior and, furthermore, how the corticotropin-releasing factor (CRF) system interferes with it (see below).

The brain CRF system

The CRF system plays a key role in a diversity of behaviors accompanying stress, anxiety, and depression and in their underlying physiology (Bale and Vale 2004; Koob and Heinrichs 1999; Reul and Holsboer 2002). The CRF system consists of four polypeptidergic ligands: CRF and urocortin (UCN) 1–3, that have different affinities for the two CRF receptor (CRF-R) types 1 and 2. CRF primarily binds to CRF-R1, due to its × 40 higher affinity over CRF-R2. While UCN1 binds to both CRF-R subtypes with equal affinity, UCN2 and UCN3 are exclusive ligands for CRF-R2 (Hsu and Hsueh 2001; Reyes et al. 2001). In addition, CRF-R gating is regulated by the CRF-binding protein (CRF-BP), which is released from astrocytes—and possibly from neurons—and binds free CRF and UCN1. Thereby, making the ligands unavailable for further receptor activation ((Behan et al. 1995), but also see (Westphal and Seasholtz 2006)). The functions of the brain CRF system range from triggering the physiological and psychological stress response to modulating emotionality and behavior including social behavior. Interestingly, the brain CRF system is also a key regulator of maternal behavior. In the maternal brain, central stimulation of either CRF-R impairs maternal care and maternal aggression, and increases anxiety as demonstrated in mice and rats (Gammie et al. 2004; Klampfl et al. 2013, 2014; Pedersen et al. 1991). Hence, a postpartum downregulation of the CRF system is necessary to avoid maternal neglect and anxiety as part of the peripartum adaptations.

Increased CRF system activity increases maternal anxiety

In general, central CRF-R activation is anxiogenic in males (Koob and Heinrichs 1999; Koob and Thatcher-Britton 1985) as well as in lactating females (Klampfl et al. 2013, 2014), while its inhibition is anxiolytic in virgin and lactating females (Klampfl et al. 2014). Interestingly, effects of CRF-R manipulation on anxiety have been localized in the BNST (Ciccocioppo et al. 2003; Davis et al. 2010; Greenwell et al. 2004; Jasnow et al. 2004; Lee and Davis 1997; Liang et al. 2001), among other brain regions, and were found to be sex-specific (Klampfl et al. 2014). In male rats, a local combined infusion of CRF with a CRF-R1, but not CRF-R2, antagonist has anxiolytic properties (Sahuque et al. 2006). However, in both virgin and lactating female rats, administration of either a selective CRF-R1 or CRF-R2 antagonist into the medial-posterior (mpBNST) (Klampfl et al. 2014), but not the anterior-dorsal part of the BNST (adBNST) (Klampfl et al. 2016a), is anxiolytic. This was confirmed by an anxiogenic effect of CRF-BP inhibition in the mpBNST, but not the adBNST (Klampfl et al. 2016b). Thus, these results support a central role of the CRF system in the mpBNST as a vital

regulator of maternal anxiety during lactation. Compellingly in humans, up to 10% of mothers suffer from maternal anxiety with negative consequences on maternal health and the mother-infant relationship (Pawluski et al. 2017).

Increased CRF system activity impairs maternal behavior

In addition to maternal anxiety, maternal care is negatively affected by a hyper-activated CRF system. This was found both centrally (Klampfl et al. 2013, 2014; Pedersen et al. 1991; Saltzman et al. 2011) and locally in the BNST, and in a stress-, receptor- and subdivision-specific manner (Klampfl et al. 2014, 2016a). Under non-stress conditions, activation of CRF-R1 in either the adBNST or the mpBNST impairs ABN and total nursing immediately. However, activation of CRF-R2 affects maternal care in a time- and behavior-specific way. In the adBNST, increased CRF-R2 signaling increases ABN, whereas it is the opposite in the mpBNST. Interestingly, in both brain regions, the effect on behavior occurs with a delay. In contrast, total nursing is reduced in both BNST subdivisions, in the adBNST immediately and in the mpBNST with a delay. On one hand, especially CRF-R1 signaling in the BNST, independent of anterior or posterior part, requires hypoactivation during lactation to guarantee adequate maternal care. On the other hand, CRF-R2 gating in the BNST postpartum appears to be rather important for fine-tuning of maternal care, and thus, might be more sensitive to environmental changes due to the opposite roles in the adBNST versus the mpBNST.

Under stressful conditions, e.g., a 10-min exposure to the stressful maternal defense test (Neumann et al. 2001), the central CRF system becomes activated resulting in impaired maternal care (Klampfl et al. 2013, 2014, 2016a). Similar to non-stressful conditions (see above), the two CRF-R subtypes have different roles depending on the subdivision of the BNST. Blockade of CRF-R1 in the adBNST as well as the mpBNST prevents the stress-induced decrease in ABN/total nursing. However, blockade of CRF-R2 signaling is only effective in the mpBNST; the stress-induced decrease in ABN returns rapidly to pre-stress levels and nursing behavior is restored. Thus, CRF-R2 appear to be more important in mediating maternal care in the mpBNST. Whereas, CRF-R1 are most likely the crucial receptor subtypes in the adBNST, especially in a stress-relevant context. Interestingly, this subdivision-specific pattern is reflected by CRF-R mRNA expression levels. While CRF-R1 mRNA is abundantly expressed throughout the BNST, CRF-R2 mRNA expression increases towards the posterior subdivisions, such as the principal nucleus of the BNST (Klampfl and Bosch, unpublished observation). However, receptor mRNA levels in the BNST are not altered in lactation compared to virgins. Therefore, on the mRNA level, the BNST receptors do not suggest a contributing role to the essential downregulation postpartum (Klampfl et al. 2014, 2016a). In contrast, CRF mRNA expression is elevated in lactating rats, in both the anterior (Klampfl et al. 2016a) and posterior BNST (Walker et al. 2001). This finding appears controversial considering the downregulated CRF system. Thus, this downregulation must happen either on a different level, e.g., the protein level or via a different target.

The CRF-BP as a regulatory protein is a potent candidate for the abovementioned downregulation during lactation. In the mpBNST, but not in the adBNST, it is indeed responsible for the restoration of maternal care following a stressful condition (Klampfl et al. 2016b). This occurs most likely by binding the endogenous ligands for CRF-R1, i.e., CRF and UCN1, and thus, decreasing CRF-R1 gating. This is partly in line with the findings described above, where CRF-R1 antagonism in the mpBNST prevents the stress-induced decrease in nursing (Klampfl et al. 2014). However, other results on the CRF-BP in the BNST are less conclusive. The CRF-BP is less abundant in the posterior than in the anterior BNST, as determined by a cross-linking assay (Klampfl et al. 2016b). Yet, CRF-BP in the adBNST seems to have no influence on maternal behavior, which is surprising given that the activation of CRF-R1 in the adBNST appears to be the main factor reducing maternal care. Thus, further studies are needed to elucidate CRF-BP's actions on the molecular level and to promote our understanding of CRF-BP during lactation with special focus on maternal care.

With respect to maternal aggression, central activation of CRF-R by either CRF or UCN1 impairs the display of aggressive behavior towards a conspecific intruder in lactating rats (Klampfl et al. 2013, 2014) and in lactating mice (D'Anna et al. 2005; Gammie et al. 2004). In support, lactating mice deficient for either CRF-R1 (Gammie et al. 2007) or CRF-R2 (Gammie et al. 2005), show less maternal aggression. These findings demonstrate species-independent effects of central CRF-R activation on maternal aggression. The picture is even more distinct when focusing on the BNST. While CRF-R1 does not modulate maternal aggression in lactating rats, CRF-R2 in the mpBNST (Klampfl et al. 2014), but not in the adBNST (Klampfl et al. 2016a), plays a significant role; CRF-R2 inhibition increases, whereas administration of UCN3 decreases this protective behavior. These findings are supported by the presence of Ucn3 mRNA (Hsu and Hsueh 2001) and fibers (Li et al. 2002) in the mpBNST, but not adBNST. The prominent effect of CRF-R2 signaling on maternal aggression in postpartum rats is in line with studies in lactating mice (D'Anna and Gammie 2009). Inhibition of CRF-R2 in the lateral septum, another key brain area for maternal aggression (Bosch 2013; D'Anna and Gammie 2009), increases aggressive behavior, whereas receptor activation reduces it. Interestingly, the lateral septum receives projections from the mpBNST (Dong and Swanson 2004), but not from the adBNST (Dong et al. 2001b). Hence, CRF-R2-related

projections from the mpBNST to the lateral septum might be involved in the display of maternal aggression.

Interactions of the brain CRF and oxytocin systems

To date, all the data on the CRF system's role in maternal behavior have shown a rather anti-maternal role for the neuropeptide system, thereby, strongly suggesting a CRF system downregulation postpartum to guarantee appropriate maternal behavior. However, it is still unclear how this regulation is brought about. Indeed, several candidate neurotransmitter systems might be involved via mutual neuromodulation. Here, the OT system is an intriguing candidate, not only because CRF-R are expressed on OT neurons and vice versa (Dabrowska et al. 2011), but also due to a recently shown modulatory role of CRF on OT release in the MPOA of lactating rats (Klampfl et al. 2018) and in the BNST of male rats (Martinon and Dabrowska 2018). To test our hypothesis, we performed microdialysis within the mpBNST to measure local OT release in response to central as well as local CRF-R manipulations in postpartum rats; this was performed based on the methodology described in a previous study investigating the impact of CRF-R manipulation on OT release in the MPOA (Klampfl et al. 2018). Furthermore, we studied whether changes of OT receptor activity affect maternal behavior following well-established protocols for local, bilateral substance infusion with subsequent behavioral testing (Bosch et al. 2010; Klampfl et al. 2014, 2016a, b, 2018). Indeed, the release of OT in the mpBNST was increased after central (Fig. 1a), but not local (Fig. 1b), CRF-R2 inhibition under both non-stress and stress conditions. This finding suggests a prominent role for CRF-R2 in regulating OT release. This is, however, not localized to the mpBNST directly. Instead, OT release in the mpBNST upon central CRF-R2 inhibition might be a secondary effect of altered CRF-R2 gating in an upstream brain region to the mpBNST. The missing effect of CRF-R2 inhibition on OT release in the mpBNST might be specific to the postpartum period or females in general as male rats show an increase of OT release following that manipulation (Martinon and Dabrowska 2018). Furthermore, acute blocking of OT receptors in the mpBNST by an OT receptor antagonist strongly impaired pup retrieval (Fig. 2). However, other parameters of maternal behavior, such as maternal care under non-stress and stress conditions, maternal aggression, and maternal anxiety were not affected by local OT receptor blockade (data not shown). The effect on pup retrieval seems to be specific to the mpBNST as OT treatment in the anterior BNST does not affect maternal motivation (Consiglio et al. 2005). The prominent effect on maternal motivation and the lack of effect on maternal aggression were rather surprising given that the MPOA, but not the BNST, is believed to be the main mediator of maternal motivation (Bosch and Neumann 2012; Numan and Stolzenberg 2009), and given the increased

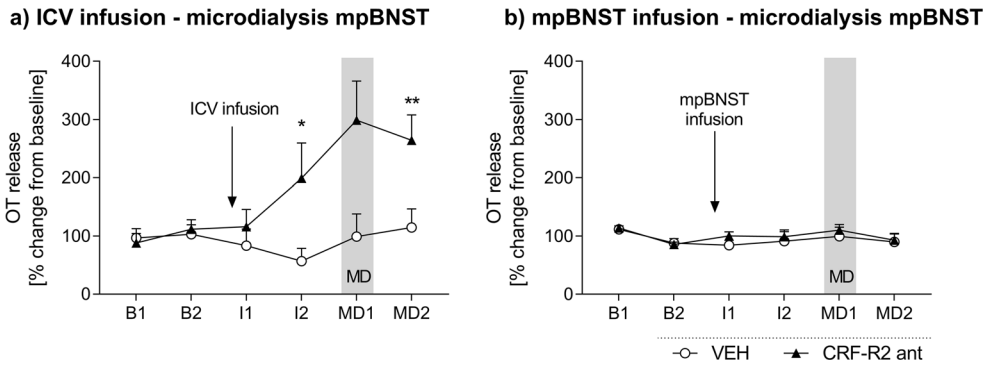

Fig. 1 Oxytocin (OT) release in the mpBNST following **a** intracerebroventricular (ICV) or **b** intra-mpBNST infusion of a CRF-R2 antagonist. For these experiments, we followed established protocols from our lab (Klampfl et al. 2013, 2014, 2018). **a** Lactating Sprague-Dawley rats were implanted with an U-shaped microdialysis probe into the mpBNST (0.7-mm posterior, + 1.5-mm lateral, 6.5-mm ventral to bregma (Paxinos and Watson 1998)) and at the same time with an ICV guide cannula (21 G, stainless-steel; 1.0-mm posterior, − 1.6-mm lateral, 1.8-mm ventral to bregma (Paxinos and Watson 1998)). **b** Lactating Sprague-Dawley rats were implanted with an U-shaped microdialysis probe that had a local infusion cannula (25 G, stainless-steel) attached for combined treatment-infusion and microdialysis-sampling in the mpBNST (for implantation coordinates see (**a**)). All surgeries were performed on lactation day 1 and experiments on lactation day 3. The probes were flushed with sterile Ringer's solution (pH 7.4; Braun, Melsungen, Germany) at a flow rate of 3.3 μl/min for 90 min, after which two basal 30-min samples were collected (B1 and B2). Afterwards, either VEH (sterile Ringer's) or the CRF-R2 subtype-specific antagonist astressin-2B (CRF-R2 ant; Sigma-Aldrich, Steinheim, Germany) were infused **a** ICV (4 μg/5 μl) or **b** into the mpBNST (4 μg/0.5 μl). Two 30-min dialysates were collected (I1 and I2) to assess drug effects on OT release. Next, the dams were subjected to the maternal defense (MD) test for 10 min and collection continued for two 30-min intervals (MD1 (including a 10-min MD test) and MD2) to assess effects of drug + stress exposure on OT release in lactating dams. Dialysates were lyophilized, and OT concentrations were measured by an OT radioimmunoassay (RIAgnosis, Sinzing, Germany). Data were analyzed using two-way ANOVA for repeated measures (factors: time × group; IBM SPSS 24.0). **a** OT release was found to significantly differ (time: $F_{5,65} = 4.03$, $p < 0.01$; group: $F_{1,13} = 9.96$, $p < 0.01$) with elevated levels at I2 ($p = 0.03$) and MD2 ($p = 0.01$) in CRF-R2 ant-treated rats compared to VEH. **b** No significant differences were found after mpBNST infusion. Data are presented as mean + SEM; $^{**}p \leq 0.01$, $^{*}p \leq 0.05$ versus VEH

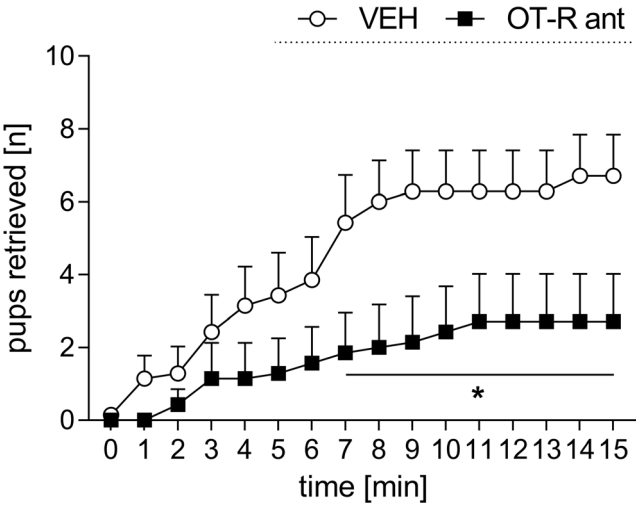

Fig. 2 Effects of oxytocin receptor (OT-R) inhibition on maternal motivation in the pup retrieval test. For this experiment, we followed established protocols from our lab (Klampfl et al. 2013, 2014, 2018). Female rats were implanted bilaterally with guide cannulas into the mpBNST (0.7-mm posterior, ± 1.5-mm lateral, 4.5-mm ventral to bregma (Paxinos and Watson 1998)) on pregnancy day 18 (Klampfl et al. 2014). On lactation day 3, dams were tested in the 15-min pup retrieval test. Briefly, 60 min prior to the test, the dams were separated from their litters and moved to a separate room. Ten minutes prior to the pup retrieval test, the dams were acutely bilaterally infused with VEH (0.5-μl sterile Ringer's solution, pH 7.4) or the OT-R antagonist (OT-R ant; des-Gly-NH2,d(CH2)5[Tyr(Me)2,Thr 4]OVT; 1 μg/0.5 μl; (Manning et al. 1989)). Eight pups of each litter were distributed in a plastic box (54 cm × 34 cm × 31 cm) with the floor covered with bedding from the home cage. The dam was placed in the middle of the box, and the time of retrieval of the pups was measured for a maximum of 15 min. When not retrieving pups, the dams mostly explored the box and displayed sniffing, rearing or self-grooming. Data were analyzed using two-way ANOVA for repeated measures (factors: time × group; IBM SPSS 24.0). The number of retrieved pups was found to be significantly different (time x× group: $F_{15,180} = 2.44$, $p < 0.01$) with OT-R ant-treated dams retrieving less pups in the 15-min test compared to VEH ($p \leq 0.05$, in each case). Data are presented as mean + SEM; *$p \leq 0.05$ versus VEH

Table 1 Effects of CRF-R1 or CRF-R2 activity in either the adBNST or mpBNST on maternal anxiety, maternal care, maternal aggression, and maternal motivation

	adBNST[a]		mpBNST[b]	
	CRF-R1	CRF-R2	CRF-R1	CRF-R2
Maternal anxiety	–	–	↑	↑
Maternal care				
Non-stress condition	↓	↓↑[#]	↓	↓
Stress condition	↓	---	---	↓
Maternal aggression	–	–	–	↓
Maternal motivation	–	–	–	–

↑, increase; ↓, decrease; —, unchanged versus VEH-treated dams

[#] Indicates a time-dependent effect, i.e., an immediate decrease and a delayed increase in maternal care

[a] Klampfl et al. Klampfl et al. 2016a

[b] Klampfl et al. Klampfl et al. 2014

OT release in the BNST during maternal aggression (Bosch 2013). Importantly, based on several drug diffusion experiments (Klampfl et al. 2014, 2016a, 2018), we can exclude that the OT receptor antagonist diffused to the adjacent MPOA, thereby inducing behavioral changes. Furthermore, in the face of mutual modulation of the OT and CRF systems in the BNST, maternal motivation is the only maternal parameter that is mediated by the OT system and at the same time the only parameter that is not mediated by the CRF system (Klampfl et al. 2014; Klampfl et al. 2016a; Klampfl et al. 2013; Klampfl et al. 2018). Here, further studies are needed to closely delineate the neuronal interactions of these two neuropeptide systems and their behavioral implications postpartum.

Conclusions

Adequate expression of maternal behavior and maternal anxiety is the result of finely tuned and balanced neurotransmitter (inter-)actions. During the postpartum period, pro-maternal neuropeptide systems are upregulated. Meanwhile, stress neuropeptide systems, such as the CRF system, are downregulated in limbic brain regions, for instance, the BNST (Table 1). While CRF-R activity requires downregulation, the CRF-BP needs upregulation to contribute to such necessary adaptations. In the BNST, different expression patterns of the CRF family members are found in the different subdivisions, contributing to a distinct impact on the various components of maternal behavior and/or maternal anxiety. As shown for the MPOA, the CRF system potentially interacts with various neurotransmitter systems to affect maternal behavior and maternal anxiety. We now have provided evidence for CRF-R2 modulating OT release in the mpBNST. Intriguingly, this novel effect following CRF-manipulation, found that OT in the mpBNST promotes maternal motivation.

In humans, a substantial dysregulation of the brain CRF system postpartum, probably together with other factors, might lead to infant neglect and even filicide (Appleby et al. 1998; Porter and Gavin 2010). Current therapeutic approaches in the treatment of underlying postpartum mood disorders are not well advanced. This could be attributed to current antidepressants and anxiolytics being developed in male-only trials. Furthermore, it has long been neglected that psychopathologies differentially evolve in females (Solomon and Herman 2009; Valentino et al. 2013). Hence, a better understanding of the mechanisms underlying postpartum mood disorders is required to advance the development of suitable treatment options.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Statement on the welfare of animals The experiments were approved by the Committee on Animal Health and Care of the local government and conformed to international guidelines on the ethical use of animals. All efforts were made to minimize the number of rats used and their suffering.

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