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REVIEW

Urocortins and their unfolding role in mammalian social behavior

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Abstract

The corticotropin-releasing factor (CRF) system is well known for its major role in coordinating the endocrine, autonomic and behavioral responses to stress. These functions have been shown to be mediated mainly by the binding of the CRF neuropeptide to its specific receptor CRFR1. Yet, the CRF system comprises several more neuropeptides, including the three urocortins, UCN1, UCN2 and UCN3, of which the latter two bind specifically to a distinct receptor—CRFR2. Unlike the brain-wide abundant expression of CRF and CRFR1, the brain expression of the urocortins and CRFR2 is rather restricted and seems to be focused in limbic areas associated with social behavior. Here, we will review accumulating evidence from recent studies that unfold the role of UCN2 and UCN3 in regulating mammalian social behavior, via activation of CRFR2.

Keywords Urocortins · Social behavior · CRF system · CRFR2 · Medial amygdala

The mammalian CRF system

The mammalian CRF system comprises two receptors—CRFR1 and CRFR2 and four ligands—CRF, Urocortin1 (UCN1), UCN2 and UCN3 (Dedic et al. 2018). CRFR1 and CRFR2 are class B, membrane bound, G protein-coupled receptors (GPCRs) (Perrin et al. 1995; Perrin et al. 1993). They share overall 70% amino acid homology, with the lowest degree of homology (40%) between their ligand-binding N-terminal extracellular domains and the highest (80%) between their transmembrane domains (Dautzenberg and Hauger 2002). These two receptors show numerous splice variants (including CRFR1α and CRFR1β and CRFR2α, CRF2β and CRFR2γ), which are expressed in various central and peripheral tissues (Henckens et al. 2016). For example, the CRF2β splice variant is expressed primarily in peripheral tissues, with the highest levels of expression in the choroid plexus of the brain, the skeletal muscle and the heart. It is also expressed in the gastrointestinal tract (Lovenberg et al. 1995b; Perrin et al. 1995) where it seems to participate in the regulation of metabolism (Kuperman and Chen 2008). Of these splice variants, CRFR1α and CRFR2α seem to be the only functional variants expressed in the rodent brain, hence will be referred to herein as CRFR1 and CRFR2. Both receptors primarily signal by Gs protein coupling, resulting in the induction of the cyclic AMP–protein kinase A (cAMP-PKA) and the extracellular signal-regulated kinase–mitogen-activated protein kinase (ERK–MAPK) pathways (Hauger et al. 2006). Yet, they also interact with other G protein systems, including $G_q\alpha$, G_i, G_o, $G_{i1/2}$ and G_z, thus activating phospholipase C variants (PLCs), resulting in the activation of ERK1 and ERK2 and an increase in intracellular Ca^{2+} concentration (Grammatopoulos et al. 2001). As for their ligands, the mature and biologically active form of CRF is a 41-amino acid peptide displaying very high (~ 1 nM) affinity (inhibitory binding constant—K_i) to CRFR1 and high affinity (~ 15 nM) to CRFR2. UCN1 is a 40-amino acid peptide with 43% amino acid homology to CRF, showing very high affinity to both CRFR1 (~ 0.3 nM) and CRFR2 (~ 1 nM). UCN2 (or stresscopin-related peptide) is a 38-amino acid peptide with 34% homology with CRF, showing low affinity (> 100 nM) to CRFR1 and very high affinity (~ 0.2 nM) to CRFR2. UCN3 (or stresscopin) is also a 38-amino acid peptide with 26% homology to CRF, which shows very low (> 1000 nM) affinity to CRFR1 and high affinity (~ 10 nM) to CRFR2 (Fekete and Zorrilla 2007). Thus, UCN2 and UCN3 are considered as selective ligands of the CRFR2 receptor (Hsu and Hsueh 2001), although this receptor may also bind CRF or UCN1.

Besides these components, the CRF system also includes two extracellular soluble proteins, the glycoprotein CRF-binding protein (CRF-BP) (Ketchesin et al. 2017) and the

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CRFR2 splice variant sCRFR2α (Chen et al. 2005), both of which bind to CRF and UCN1 with nanomolar-range affinities, hence may function to control the availability of the latter two neuropeptides to their functional receptors. Interestingly, CRF-BP was suggested to function also as a GPCR escort protein regulating the trafficking of CRFR2 proteins to the cell membrane (Slater et al. 2016).

The functions of the mammalian CRF system

Despite the common conception that the activation of the hypothalamic pituitary adrenal (HPA) axis is the most fundamental function of the CRF system, an evolutionary examination of how this system evolved shows just the opposite (Lovejoy et al. 2014). The system exists in mollusks and insects in a form of a single ligand–single receptor system with a main function of controlling diuresis (osmoregulation). The divergence of both ligands and receptors occurred during vertebrate evolution. A first genome duplication in early vertebrates created two ligands—the CRF/UCN1 and UCN2/UCN3 paralogs and two receptors—CRFR1 and CRFR2. At this evolutionary stage, the system started to expand its function beyond osmoregulation. Thus, in early vertebrates, the CRF system is also involved in controlling energy balance, in the form of regulating nutrient acquisition, digestion and energy utilization. Accordingly, while the CRFR1 retains much of its osmoregulatory role, CRFR2, through the action of its specific ligand UCN2/UCN3, became more and more associated with controlling energy balance, via regulation of metabolic rate, appetite and feeding behavior (Kuperman and Chen 2008; Richard et al. 2002). Then, a second genome duplication separated between CRF and UCN1 and between UCN2 and UCN3, thus generating the form of the CRF system found in modern vertebrates, with four ligands and two receptors. Only at this stage did the CRF-CRFR1 ligand-receptor couple become specialized in controlling the HPA axis, which developed in modern vertebrates (Denver 2009). Accordingly, the vertebrate CRF appears to be most distant from the ancestral peptides (Lovejoy 2009). Therefore, considering CRF itself as the prototypical ligand of the CRF system is misleading.

CRF is most abundant in the paraventricular nucleus of the hypothalamus (PVN), from where it regulates the HPA axis. This complex hypothalamic nucleus harbors multiple populations of magnocellular and parvocellular neurons expressing various neuropeptides, including CRF, thyrotropin-releasing hormone (TRH), oxytocin and arginine-vasopressin (Swanson and Sawchenko 1983). Parvocellular neurons of this nucleus project via the external zone of the median eminence and release CRF into the hypophyseal portal vasculature, which transports the neuropeptide to corticotropic cells of the anterior pituitary. The activation of CRFR1 expressed by these cells stimulates the release of the adrenocorticotropic hormone (ACTH) and other pro-opiomelanocortin (POMC)—derived peptides from the anterior pituitary (Vale et al. 1983). ACTH, in turn, triggers the synthesis and release of glucocorticoids from the adrenal cortex (cortisol in humans, corticosterone in rodents), which mediate various physiological, endocrine and metabolic reactions and ultimately prepare the organism for a stressful situation (Joels and Baram 2009). Moreover, CRF also regulates the sympathetic stress response by acting on the locus coeruleus, adrenal medulla and peripheral sympathetic nervous system (Brown et al. 1982). Thus, CRF plays a crucial part in coordinating the peripheral stress response systems and the central release of noradrenaline in reaction to stressful challenges (Turnbull and Rivier 1997). It should be noted that stress may be defined as a state in which homeostasis is actually threatened or perceived to be so (Chrousos 2009); therefore, it is not necessarily associated with aversive situations. In fact, the physiological stress response to appetitive, rewarding stimuli can be as large as the response to a negative stimulus. For example, in rats, positive experiences such as sexual encounters and social victory induce a similar degree of HPA axis activation as an aversive footshock or social defeat (Koolhaas et al. 2011).

Brain expression of urocortins and their receptors

Beyond the PVN, CRF is abundantly expressed throughout the brain (Merchenthaler et al. 1982; Sawchenko and Swanson 1985). In the mammalian brain, CRF-expressing neurons are found in hypothalamic and extrahypothalamic regions, including the olfactory bulb, bed nucleus of the stria terminalis (BNST), medial preoptic area, lateral hypothalamus, central nucleus of amygdala, geniculate nucleus, Barrington's nucleus, dorsal motor complex, inferior olive nucleus and throughout the cerebral cortex. The broad distribution of CRF neurons suggests many functions of the peptide beyond HPA axis regulation (Turnbull and Rivier 1997).

In contrast to the brain-wide expression of CRF, other urocortins are expressed in a very restricted number of brain areas. In rodents, UCN1 is mainly expressed in the centrally projecting Edinger-Westphal nucleus (EWcp) and sparsely distributed in the lateral superior olive, supraoptic nucleus (SON), and hypoglossal nuclei (Bittencourt et al. 1999; Vaughan et al. 1995). UCN2 is expressed in the PVN, SON, arcuate nucleus and locus coeruleus (Reyes et al. 2001), whereas UCN3 is mainly expressed in the medial amygdala (MeA), rostral perifornical area of the hypothalamus, BNST, superior

paraolivary nucleus, nucleus parabrachialis and the premammillary nucleus (Deussing et al. 2010; Lewis et al. 2001). The rather focused brain distribution of the urocortins, as compared to CRF, suggest that their activity is largely restricted to relatively specific brain functions.

The brain distribution of the CRF/urocortins receptors CRFR1 and CRFR2 goes hand in hand with this suggestion. Besides its high expression in the anterior pituitary, CRFR1 mRNA is abundantly expressed throughout the brain, with high levels in the cerebellum and neocortical, limbic, midbrain and brainstem regions, moderate levels in the dorsal and median raphe nuclei and low levels in the PVN (Van Pett et al. 2000). In contrast, CRFR2, which binds most specifically to UCN1, UCN2 and UCN3, shows a much more restricted distribution pattern that is virtually confined to subcortical, mainly limbic structures. The highest levels of CRFR2 mRNA are found in the lateral septum (LS) and the ventromedial hypothalamic nucleus (VMH), whereas moderate levels are evident in the olfactory bulb, nuclei of the extended amygdala, hippocampus, PVN and SON, inferior colliculus and raphe nuclei (Chalmers et al. 1995; Van Pett et al. 2000).

The restricted distribution of CRFR2 and its specific ligands, UCN1, UCN2 and UCN3, in mostly limbic areas (see Table 1), as compared to the brain-wide distribution of CRF and CRFR1, suggests that while the CRF-CRFR1 ligand-receptor system deals mainly with general stress, the three urocortins and CRFR2 create a system designated to more specific functions. For example, the uniquely high expression level of UCN1 in EWcp neurons, which are bidirectionally connected to multiple hypothalamic nuclei associated with metabolism (da Silva et al. 2013; Dos Santos Junior et al. 2015), was suggested to connect between stress and metabolism (Xu et al. 2012). Another possibility is that UCN1 expression in the EWcp is connected to the central role of this nucleus in the regulation of arousal and attention (Li et al. 2018; Lovett-Barron et al. 2017). Since many of the CRFR2-expressing brain areas were recognized as having a unique role in mammalian social behavior (Adolphs 2009; Goodson and Kabelik 2009; Young 2002), it was recently suggested that the UCN2/3-CRFR2 system may play a role in regulating social behavior (Hostetler and Ryabinin 2013). This notion is further supported by studies showing that knocking out UCN2 or UCN3 in mice does not produce overt effects on stress reactivity but does alter social behavior (Breu et al. 2012; Deussing et al. 2003). As the role of the CRF system in stress was recently described in several excellent review papers (see for example Dedic et al. 2018; Henckens et al. 2016), we will focus in this review on the possible role of the CRFR2/UCN2-3 system in several aspects of mammalian social behavior. It should be noted that this role may significantly differ between males and females.

Table 1 Main brain areas harboring a significant number of neurons expressing the various urocortins or the CRFR2 receptor, arranged from rostral to caudal according to their brain location (Chalmers et al. 1995; Deussing et al. 2010; Lewis et al. 2001; Reyes et al. 2001; Van Pett et al. 2000)

Brain area	UCN1	UCN2	UCN3	CRFR2
Telencephalon				
Olfactory bulb				X
Lateral septum				X
Medial septum				X
Bed nucleus of stria terminalis			X	X
Medial amygdala			X	X
Cortical amygdala				X
Basolateral amygdala				X
Central amygdala				X
Hippocampus				X
Entorhinal cortex				X
Diencephalon				
Medial preoptic area			X	
Paraventricular nucleus	X	X		X
Supraoptic nucleus	X	X		X
Lateral hypothalamic area				X
Ventromedial hypothalamus				X
Perifornical area			X	
Arcuate nucleus		X		
Mesencephalon				
Edinger-Westphal nucleus	X			
Superior colliculus				X
Inferior colliculus				X
Interpeduncular nucleus				X
Periaqueductal gray	X			X
Pons/medulla				
Raphe nuclei				X
Ventral tegmental area				X
Parabrachial nucleus			X	
Superior paraolivary nucleus			X	
Lateral superior olive	X		X	
Locus coeruleus		X		
Facial nucleus	X	X		
Ambiguous nucleus	X			
Spinal trigeminus nucleus	X	X		
Hypoglossal nucleus	X	X		
Nucleus of solitary tract				X

Social isolation

The survival of members of social species is threatened when they are socially isolated. Therefore, social isolation is considered to be a significant social stressor in rodents, as well as in humans. This is most acutely seen in the separation of infants from their mothers, which in many mammalian species leads

to an immediate increase in distress vocalizations of the infants (Shair 2007). Yet, social isolation has also been shown to be highly stressful in adulthood (Fone and Porkess 2008). Much evidence links social isolation-induced stress to the brain serotonergic system (Fone and Porkess 2008; Marsden et al. 2011; Muchimapura et al. 2003; Soga et al. 2015). Interestingly, CRFR2 is abundant in the midbrain raphe nuclei, the main source of serotonergic innervation in the brain (Lovenberg et al. 1995a; Van Pett et al. 2000). Accordingly, central (Staub et al. 2005) or local (Hale et al. 2010) administration of UCN2 in both rats and mice induced enhanced expression of cFos, a well-established immediate-early gene marker of neuronal activity, in subpopulations of serotonergic neurons of the dorsal raphe nucleus. Multiple studies suggest that this CRFR2-mediated modulation of the brain serotonergic system plays a role in the response of rodents to social isolation. Isolation rearing of juvenile male rats caused increased CRFR2 expression in the raphe nuclei (Lukkes et al. 2009b), which may promote the isolation-induced increase in social anxiety, revealed in these animals by the plus-maze test (Lukkes et al. 2009a). Accordingly, this anxiogenic effect of social isolation in juvenile male rats could be prevented by blocking CRFR2 but not CRFR1, specifically in the raphe nuclei (Bledsoe et al. 2011). On the other hand, CRFR2-knockout (KO) male mice exhibited impaired adaptation to isolation stress as evidenced by prolonged hypophagia and associated weight loss (Coste et al. 2006). These results suggest that CRFR2 receptors in the brain also contribute to the habituation of social stress responses.

In the monogamous prairie voles (*Microtus ochrogaster*), who typically rear offspring biparentally, an increase in CRFR2 expression in the raphe nuclei was observed in female pups reared without a father (Ahern and Young 2009). On the other hand, in adult female prairie voles, CRFR2 mRNA levels were downregulated in both the hypothalamus and hippocampus following chronic (4 weeks) social isolation (Pournajafi-Nazarloo et al. 2009).

Altogether, these results suggest a complex involvement of CRFR2 receptors located in distinct brain regions in the response to social isolation.

Social defeat

Social defeat is an ethologically relevant model of social stress, with strong translational and construct validity (Wood et al. 2012). In this paradigm, mostly used with males, an intruder subject is placed in the home-cage of a larger dominant aggressive conspecific (resident). Following an attack by the resident, which in most cases defeats the intruder in just a few minutes, the intruder is placed for a while behind a mesh screen that protects it from further direct attacks while maintaining the social stressor. Following this procedure with Wistar rats, Fekete et al. (2009) found a 5–10-fold increase in cells expressing cFos, specifically in the arcuate nucleus, VMN and the MeA. In the latter brain area, which is known to express high levels of CRFR2 (Van Pett et al. 2000), a significant co-localization was observed between the expression of cFos protein and CRFR2 mRNA. These results suggest a strong involvement of CRFR2-expressing MeA cells in the response to social defeat.

The Syrian hamster (*Mesocricetus auratus*) is a species, the males of which typically demonstrate a very high level of territorial aggression but turn to a defensive strategy following a single social defeat. This unique conditioned social defeat model was used and reported by Cooper and Huhman in a series of papers (Cooper and Huhman 2005, 2007, 2010) to evaluate the effects of various antagonists of the CRFR1 and CRFR2 receptors. They found that intracerebroventricular (ICV) application of the CRFR2-specific antagonist antisauvagine-30 but not the CRFR1-specific antagonist CP-154,526, prior to the social defeat training, significantly reduced the conditioned defeat response. This effect was recapitulated by local application of CRFR2 antagonist but not the CRFR1 antagonist, to the BNST, suggesting involvement of this brain area. Interestingly, local application of the CRFR2 antagonist to the raphe nuclei inhibited only the flight response of the defeated animals, suggesting again that CRFR2 receptors located in distinct brain areas play different roles in social stress responses.

Aggression

Inter-male aggression is often studied using the resident-intruder paradigm, in which a non-aggressive unfamiliar male is put into the home-cage of a subject conspecific (resident), who became highly aggressive following several weeks of social isolation (Miczek 1979). Interestingly, there is a significant difference in this type of aggression, between UCN2-deficient and CRFR2-deficient mice. While UCN2-deficient mice showed reduction in aggression during the resident-intruder paradigm (Breu et al. 2012), CRFR2-deficient mice showed no such reduction (Gammie et al. 2005). However, when placed in a novel environment, CRFR2-deficient mice displayed elevated aggression towards other males. This discrepancy between the two genetically modified mouse lines may be due to the activity of the other urocortins (UCN1, UCN3) that may activate CRFR2 receptors in UCN2-deficient mice.

Maternal aggression is another ethologically relevant paradigm of social stress, usually tested in lactating dams by removing the pups from the home-cage and introducing a non-aggressive male instead. Using this paradigm, D'Anna et al. (2005) found that ICV administration of either UCN1 or UCN3 to lactating female mice reduced their maternal

aggression. These treatments, however, did not affect pup retrieval behavior, which test for maternal care. Interestingly, both neuropeptides increased cFos expression in the LS and BNST, two areas well known to be involved in aggression and to express high levels of CRFR2. In a later study (D'Anna and Gammie 2009), this group reported that local application of either CRF, UCN1, or UCN3 to the LS reduced maternal aggression but did not reduce maternal care, in a CRFR2-dependent manner. These results suggest an involvement of CRFR2 in the regulation of maternal aggression. Nevertheless, the same group also found (Gammie et al. 2005) that CRFR2-KO mice exhibit reduced maternal aggression, with no change in pup retrieval, thus suggesting an opposite effect of CRFR2 on maternal aggression. This discrepancy may be due to developmental effects of the genetic CRFR2 knockout. In a more recent paper, Klampfl et al. (2014) showed in rats that infusion of CRFR2 agonist but not CRFR1 agonist, to the BNST abolishes maternal aggression, further supporting the role of CRFR2 expression in the LS and BNST for this specific type of social stress response.

Social interaction and memory

The common denominator of all aforementioned paradigms is that they all test for various types of social stress. However, social stress is just one amongst many aspects of the relations between the individual and its social environment. Individuals of social species regularly encounter conspecifics with whom they interact and collaborate. Such social relationships require the ability to tell, memorize and recognize specific individuals for extended periods. This ability, termed social recognition memory (SRM), is usually tested in mice and rats by their innate tendency to investigate novel social stimuli more persistently than familiar ones. Thus, the social recognition paradigm (Thor and Holloway 1982) principally comprises two short (2–5 min) unrestricted encounters of the subject with the same social stimulus, separated by a certain time interval. A reduction in the time spent by the subject on investigating the social stimulus (investigation time) in the second encounter, as compared to the first one, is considered to reflect SRM. This type of memory is also tested by the social discrimination paradigm in which, following an encounter with a social stimulus, the subject is simultaneously exposed to the same stimulus and to a novel one (Engelmann et al. 1995). Normally, the subject explores the novel stimulus for more time than the familiar one, reflecting the recognition of the familiar social stimulus. Thus, testing for SRM actually involves three distinct behavioral constructs: the motivation for social interaction per se, termed sociability, the preference for novel social stimuli, termed social novelty preference and the social memory itself (SRM). Interestingly, these three constructs seem to be separated by the effects of the various urocortins on the social behavior of rodents. Central administration of CRF, UCN1 and the CRFR1 agonist stressin1-A reduced the tendency of male rats for social interactions in a CRFR1 dependent manner (Dunn and File 1987; Gehlert et al. 2005; Sajdyk et al. 1999; Zhao et al. 2007). Similar results were reported in male mice (Bagosi et al. 2017b). In contrast, no clear effect of UCN2 or UCN3 on the tendency for social interaction by either male or female mice was reported in multiple studies (Breu et al. 2012; Deussing et al. 2010; Zhao et al. 2007). Moreover, both CRF and UCN1 but not UCN2 and UCN3, were found to reduce social novelty preference in male mice (Bagosi et al. 2017a). Yet, when it comes to social memory, it seems as if UCN3 is the most influential of all urocortins. Deussing et al. (2010) found that both UCN3-deficient mice and CRFR2-deficient mice exhibited improved SRM, as reflected by their ability to discriminate a familiar stimulus from a novel one after a longer time interval from the first exposure to the familiar stimulus, as compared to WT littermates. However, no effect of the genetic modification was observed in the object recognition test, suggesting that the improved memory is restricted to social stimuli. In contrast, UCN2-deficient mice did not show any change in their SRM. These results suggested, for the first time, that the UCN3-CRFR2 ligand-receptor system may be specifically involved in the regulation of social memory. This is especially interesting as both these proteins are expressed in the MeA, a brain area well known for its role in social memory (Maroun and Wagner 2016). Blockade of protein synthesis in the MeA abolished long-term SRM in male rats (Gur et al. 2014), demonstrating the importance of synaptic plasticity in this brain area for social memory. Notably, the MeA is known as a center for neuroendocrine control of social behavior, as it expresses many receptors and ligands of the neuroendocrine system (Frankiensztajn et al. 2018). Specifically, the activity of the neuropeptide oxytocin, solely produced in the hypothalamic PVN and SON nuclei, in the MeA was shown to be crucial for social memory (Ferguson et al. 2001). The connection between the UCN3-CRFR2 dyad in the MeA and social memory was recently examined by two distinct studies using very different methodologies.

In the first study (Frankiensztajn et al. 2018), the authors used in vivo electrophysiological recordings in anesthetized male rats to show that oxytocin induces depression of synaptic inputs arriving to the MeA from the accessory olfactory system, which are thought to mediate social recognition in rodents. Then they found that the central administration of UCN3 before oxytocin application reverses the effect of oxytocin on synaptic plasticity in the MeA from long-term depression (LTD) to long-term potentiation (LTP). This effect of UCN3 was dependent on oxytocin activity, as UCN3 application alone did not change the synaptic plasticity in the MeA. Thus, UCN3 seems to modulate the effect of oxytocin on synaptic plasticity in the MeA, turning it from LTD to LTP

(see Fig. 1a, b). Notably, this effect was CRFR2-dependent, as it could be completely blocked by the CRFR2 antagonist (Frankiensztajn et al. 2018). Finally, when the social recognition test was used with male rats, ICV administration of oxytocin and UCN3 had opposite effects on SRM: while oxytocin seems to enhance it, compared to saline

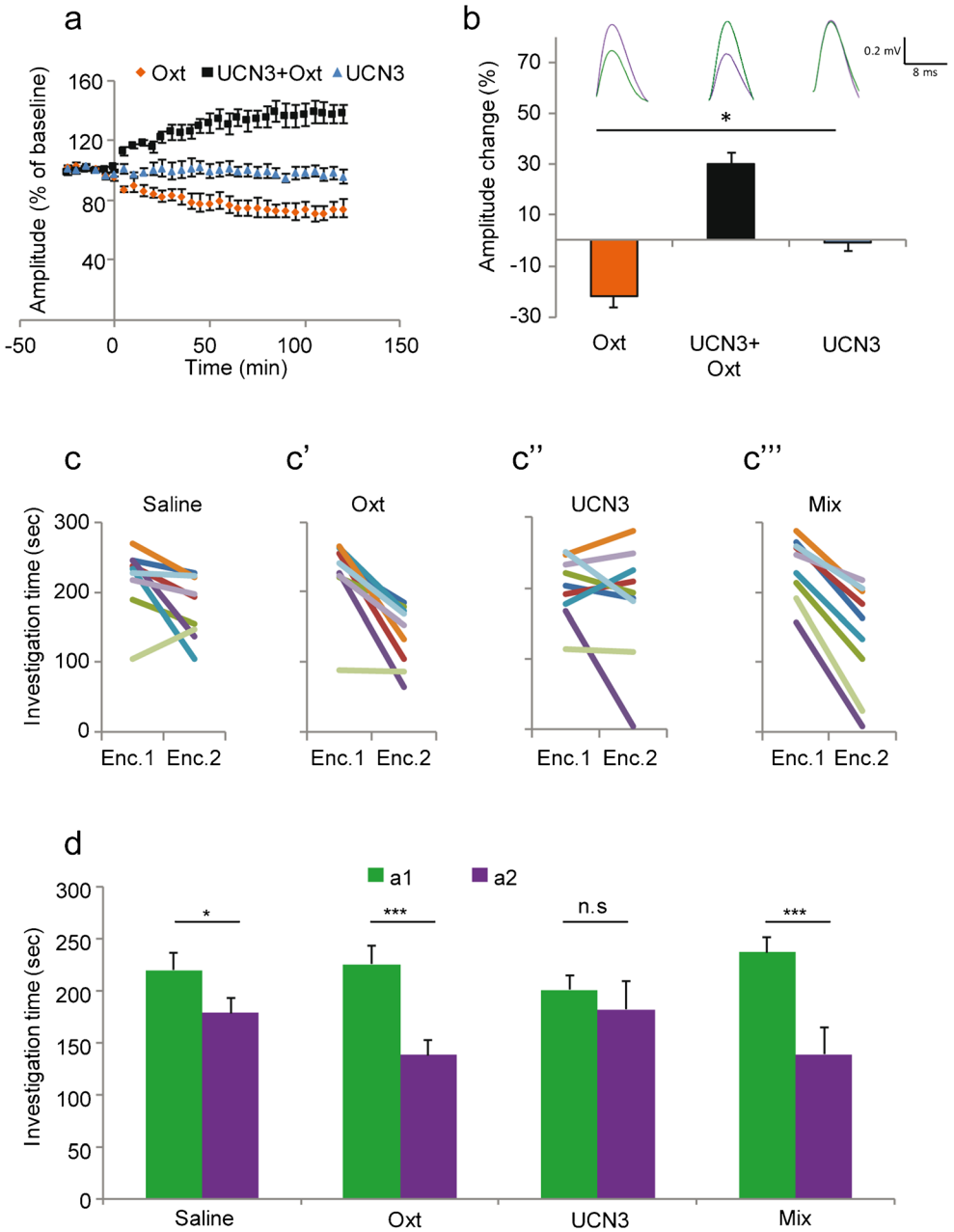

Fig. 1 UCN3 turns the oxytocin-dependent LTD in the MeA into LTP and blocks social recognition memory. (a) Mean amplitude (% of baseline) of the MeA evoked field potential (EFP) response to AOB stimulation after theta-burst stimulation (TBS) given at time 0, following oxytocin (Oxt) administration (orange diamonds, $n = 6$), UCN3 (blue triangles, $n = 6$) or oxytocin and UCN3 (black squares, $n = 9$). (b) Mean percent amplitude changes from baseline (± SEM) of the EFP signals recorded in **a**, averaged over 120 min after TBS. Representative traces of the response before (purple) and 120 min after (green) TBS are shown superimposed below each bar. Note the transition from LTD with oxytocin alone to LTP with oxytocin and UCN3 administration (one-way ANOVA; $F = 34.501$, $df = 2.18$, $p < 0.00005$; $^*p < 0.05$, Bonferroni's post hoc). (c-c‴) Lines connecting the time spent by each adult subject ($n = 9$ adult male SD rats, colored differently for each rat) to investigate a juvenile SD male during their first (Enc. 1) and second (Enc. 2) 5-min encounters, following ICV injections of either saline (c), oxytocin (Oxt, 1 ng, c′), UCN3 (50 ng, c″) or a mixture of UCN3 and the CRFR2-specific antagonist antisauvagine-30 (4 μg, Mix, c‴). (d) Mean (+ SEM) values of the results shown in c-c‴. Social recognition memory was determined by a statistically significant difference in paired t test between the two encounters ($^*p < 0.05$, $^{***}p < 0.001$). Note that UCN3 administration is the only condition where SRM loss was observed

administration, UCN3 abolished it. SRM abolishment by UCN3 was CRFR2-dependent, as it was blocked when the CRFR2 antagonist antisauvagine-30 was applied in mixture with UCN3 (Fig. 1c, d). Altogether, these results suggest a role for CRFR2-mediated UCN3 activity in the MeA in the regulation of social memory via its interaction with oxytocin. Such interaction between CRFR2 and oxytocin was recently demonstrated in the rat BNST, where oxytocin-expressing axon terminals were demonstrated to express CRFR2 (Dabrowska et al. 2011) and activation of these receptors was found to inhibit oxytocin release (Martinon and Dabrowska 2018). Another example of negative interaction between the two neuropeptides was recently reported in male prairie voles, where suppression of oxytocin signaling in the nucleus accumbens by chronic activation of CRFR2 was demonstrated to be involved in the induction of an aversive emotional state following partner loss (Bosch et al. 2016).

In the second study (Shemesh et al. 2016), the authors elegantly used a battery of a genetically modified mouse line to explore the role of UCN3 and CRFR2 in social behavior. First, they demonstrated that both UCN3-KO and CRFR2-KO male mice display deficits in social novelty preference. These mice showed a reduced tendency to explore a novel conspecific while exhibiting an increased tendency to explore their familiar littermates. These deficits seem to be due to the activity of both proteins in the MeA, as a local shRNA-mediated downregulation of CRFR2 expression in the MeA caused the same deficits. Moreover, the local application of UCN3 to the MeA, as well as optogenetic stimulation of UCN3-expressing MeA neurons, caused exactly opposite effects as compared to the genetic elimination of the gene, that is, increasing the tendency of the animals to explore novel social stimuli and reducing their interest in familiar ones. The authors then explored the identity and connectivity of the MeA cells expressing each gene and found that UCN3-expressing cells are bilaterally connected with the BNST and PVN, both of which are important nodes of the mammalian social brain network (Goodson and Kabelik 2009). CRFR2 neurons were found to be a distinct population of GABAergic neurons, which also innervate the PVN and BNST, in addition to the LS and the paraventricular nucleus of the thalamus (PVT). Thus, both populations of MeA neurons have close synaptic ties with other areas of the "social brain," further supporting their involvement in the regulation of social behavior.

Conclusions and perspectives

While the role of the mammalian CRF-CRFR1 ligand-receptor system in the regulation of stress responses is very well established and studied, much less is known about the role of the urocortins UCN2 and UCN3 and their preferred receptor CRFR2. The highly restricted brain expression of these members of the CRF system in limbic areas associated with social behavior, as opposed to the brain-wide expression of CRF and CRFR1, suggests that besides their role in metabolism regulation via peripheral tissues, urocortins play a significant role for the regulation of social behavior via their brain activity. It should be noted that metabolism, social behavior and stress are highly interconnected both physiologically and behaviorally. Therefore, it may not be surprising that the CRF system seems to be deeply involved in these three functions, which are crucial for the survival of the individual. Here, we reviewed a series of recent works exploring the involvement of UCN2 and UCN3, via the CRFR2 receptor in the regulation of mammalian social behavior, which started to reveal the underlying mechanisms. Future studies, using contemporary methodologies such as genome editing, optogenetics, viral-vector mediated neuronal tracing and single-cell transcriptomics, should be used in the near future to clarify the intricate anatomical and functional networks underlying the multiple interconnected actions of urocortins in the brain.

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