Brain Research 1663 (2017) 114–122

Contents lists available at ScienceDirect

Brain Research

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

Research report

The effects of CRF and urocortins on the sociability of mice

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ARTICLE INFO

Article history:
Received 7 December 2016
Received in revised form 2 March 2017
Accepted 3 March 2017
Available online 14 March 2017

Keywords:
CRF
Urocortins
Sociability
Mice

ABSTRACT

The aim of our study was to determine the role of corticotropin-releasing factor (CRF), the urocortins (Ucn1, Ucn2 and Ucn3) and their receptors (CRF_1 and CRF_2) in the sociability of mice. Male CFLP mice were administered intracerebroventricularly (icv) with CRF and urocortins alone or in combination with antalarmin (specific CRF_1 antagonist) and astressin$_{2B}$ (specific CRF_2 antagonist) and then investigated in a Crawley social interaction test arena, that consists of three chambers. An unknown male in a cage was put in the first chamber and an empty cage was put in the opposite chamber. The tested male was habituated with the middle chamber for 5 min and then allowed to explore the remaining chambers for 5 min, during which the number of entries and the time of interaction were measured. Intracerebroventricular administration of CRF decreased significantly the number of entries and the time of interaction with the unknown male and these effects were blocked by antalarmin, but not astressin$_{2B}$. In contrast, central administration of Ucn1 increased significantly the number of entries into the chamber of the unknown male, without changing the time of interaction and this effect was blocked by astressin$_{2B}$, but not antalarmin. Central administration of Ucn2 and Ucn3 didn't influence remarkably the number of entries, but it reduced the time of interaction between the male mice. Our study suggests that CRF and Ucn1 may play important, but different roles in sociability, and that Ucn2 and Ucn3, playing similar roles, must be also involved in social interactions.

1. Introduction

Corticotropin-releasing factor (CRF) is an important neuropeptide isolated from the brain that regulates the responses to stress (Vale et al., 1981). These responses are endocrine, represented by the activation of the hypothalamic-pituitary-adrenal (HPA) axis that is reflected by the elevation of concentration of the plasma adrenocorticotropic hormone (ACTH) and glucocorticoids which on their turn inhibit the CRF release; autonomic, such as increase of the blood pressure, increase of the heart rate and vasoconstriction which are mediated by catecholamines released from the adrenal medulla; metabolic, such as glycogenolysis, gluconeogenesis, lipolysis and proteolysis which are mediated by glucocorticoids released from the adrenal cortex; and behavioral, such as inhibition of reproduction, increased locomotion in a familiar environment, decreased locomotion in an unfamiliar environment etc. (Carrasco and Van de Kar, 2003). Besides these effects, CRF has been demonstrated to participate to social interactions indirectly, as a hypothalamic neurohormone stimulating the HPA axis (Backstrom and Winberg, 2013; Hostetler and Ryabinin, 2013) but also directly, as an extrahypothalamic neurotransmitter promoting behavioral actions (Lim et al., 2007; Sajdyk et al., 1999).

The actions of CRF are mediated by two distinct receptors: CRF_1 receptor and CRF_2 receptor (Chang et al., 1993). These receptors belong to the class B subtype of G protein-coupled receptors (GPCRs) and, like all GPCRs, consist of an amino-terminal extracellular region, a carboxyl-terminal intracellular tail and seven, transmembrane segments, connected by alternating intracellular and extracellular loops (Grammatopoulos et al., 2001). There is nearly 70% identity between CRF_1 and CRF_2 receptors at the amino acid level with the transmembrane and intracellular domains of the CRF receptors presenting the highest homology (over 80% identity) (Grammatopoulos et al., 2001). A third receptor, CRF-binding protein (CRF-BP) was also isolated in the brain and the pituitary gland and is believed to modulate the endocrine activity of CRF (Behan et al., 1995). CRF_1 and CRF_2 receptors are believed to have antagonistic roles in the CNS. Activation of the CRF_1 seemed to induce stimulation of the HPA axis, anxiety, depression, decreased appetite and increased colonic movements, inflammation (Dautzenberg and Hauger, 2002). In contrast, activation of the CRF_2 seemed to produce anxiolytic and antidepressant effects, decrease of food intake and gastric emptying, vasodilation,

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http://dx.doi.org/10.1016/j.brainres.2017.03.003
0006-8993/

cardioprotection (Dautzenberg and Hauger, 2002). As regards the role of CRF_2 in the regulation of the HPA axis, it remains controversial due to different results obtained in rats and mice (Jamieson et al., 2006; Maruyama et al., 2007; Pelleymounter et al., 2004).

Since CRF was isolated (Vale et al., 1981), a growing family of CRF-like peptides have been discovered. Today the mammalian members of this family include CRF, urocortin 1 (Ucn1) (Vaughan et al., 1995), urocortin 2 (Ucn2), also known as stresscopin-related peptide (SRP) (Reyes et al., 2001), and urocortin 3 (Ucn3), also known as stresscopin (Scp) (Lewis et al., 2001). The name urocortin derives from the fish homologue urotensin (63% sequence identity) and the mammalian analogue corticotropin (45% sequence identity) (Skelton et al., 2000). These CRF-like peptides share common amino acidic elements, but differ in their pharmacological properties. Thus, CRF binds preferentially to CRF_1, Ucn1 attaches equipotently to both CRF receptors, whereas Ucn2 and Ucn3 bind selectively to CRF_2. In addition, CRF and Ucn1 can be found attached to CRF-BP (Fekete and Zorrilla, 2007). Many physiological functions were attributed to the urocortins, including regulation of stress response (Suda et al., 2004), modulation of food intake and satiety (Richard et al., 2002), gastrointestinal motility (Martinez et al., 2002), vasodilation and cardioprotection (Takahashi et al., 2004), and recently, social interaction (Breu et al., 2012; Deussing et al., 2010).

Previous findings regarding the possible role of CRF and CRF-related peptides in social behavior of different species have been reviewed in two recent studies (Backstrom and Winberg, 2013; Hostetler and Ryabinin, 2013). Despite that the primary focus in these studies has been on the effects of social stressors, such as social defeat and social isolation on the CRF system (Backstrom and Winberg, 2013; Carpenter et al., 2009), there have been also insights on the role of CRF system in prosocial and affiliative behaviors, such as parental care, maternal defense, sexual behavior and pair bonding (Carpenter et al., 2014; Hostetler and Ryabinin, 2013). The aim of our study was to investigate the effects of the central administration of CRF and the urocortins on the sociability of mice.

2. Results

Central administration of CRF decreased significantly the number of entries ($F(3,23) = 8.802$; $p = 0.0005$) and the time of interaction ($F(3,23) = 8,942$; $p = 0.0004$) with the unknown mouse ($F(3,23) = 16.17$; $p < 0.0001$) and the unknown object ($F(3,23) = 11.84$; $p < 0.0001$) and consequently, the total number of entries ($F(3,23) = 18.61$; $p < 0.0001$) and the total time of interaction ($F(3,23) = 20.21$; $p < 0.0001$), compared to the controls (Figs. 1 and 2). The selective CRF antagonists antalarmin and $astressin_{2B}$ administered alone did not alter significantly any of these parameters ($p < 0.05$) (Figs. 1 and 2). The inhibitory effects produced by CRF were reversed by antalarmin ($p < 0.05$), but not $astressin_{2B}$ ($p > 0.05$) (Figs. 3 and 4).

Central administration of Ucn1 increased significantly the number of entries into the chamber of unknown mouse ($F(3,31) = 10.24$; $p < 0.0001$) and accordingly the total number of entries ($F(3,31) = 11.09$; $p < 0.0001$), without influencing significantly the number of entries into the chamber of the unknown object ($F(3,31) = 0.8287$; $p = 0.4882$). Ucn1 did not influence the time of interaction with the unknown male ($F(3,31) = 10.74$; $p > 0.05$) and the unknown object ($F(3,31) = 0.9611$; $p = 0.4234$) and consequently the total time of interaction was not altered either ($F(3,31) = 5.153$; $p = 0.0052$), compared to the controls (Figs. 5 and 6). The significant effect on the number of entries and the non-significant effect on the time of interaction induced by Ucn1 were reduced remarkably by both antalarmin ($p > 0.05$) and $astressin_{2B}$ ($p < 0.05$) (Figs. 7 and 8). Central administration of Ucn2 and Ucn3 did not change significantly the number of entries into any of the chambers ($F(3,31) = 5.153$; $p = 0.0052$, $p > 0.05$), but both peptides reduced the time of interaction between the males ($p < 0.05$). This reduction was observed only in case of the time spent with the unknown mouse ($F(3,31) = 10.74$; $p < 0.0001$) and the total time of interaction ($F(3,31) = 11.09$; $p < 0.0001$), but not the time spent with the unknown object ($F(3,31) = 0.9611$; $p = 0.4234$) (Figs. 5 and 6).

The dose-response curves indicated no significant changes in the horizontal and the vertical activities of the mice tested in parallel in a conducta system, following icv injection of 0.5-1-2-5 μg/2 μl of CRF, Ucn1, Ucn2 or Ucn3 (Figs. 9–12).

3. Discussion

Based on these results, we suggest that CRF and Ucn1 may play important, but different roles in sociability, and that Ucn2 and

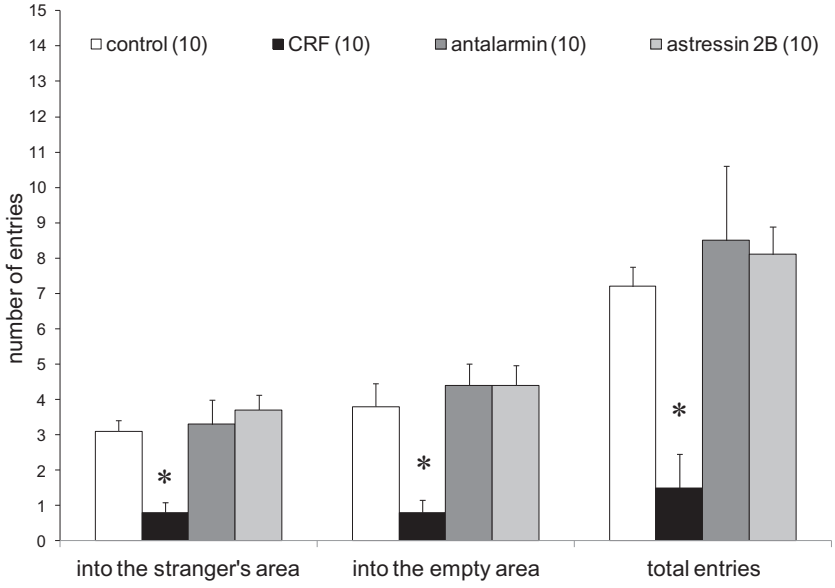

Fig. 1. The effects of CRF, antalarmin and $astressin_{2B}$ on the number of entries in the three chamber test investigating the sociability of mice. Values are presented as means ± SEM; statistically significant difference was accepted for $p < 0.05$ and indicated with * for CRF or antalarmin or $astressin_{2B}$ *vs.* control.

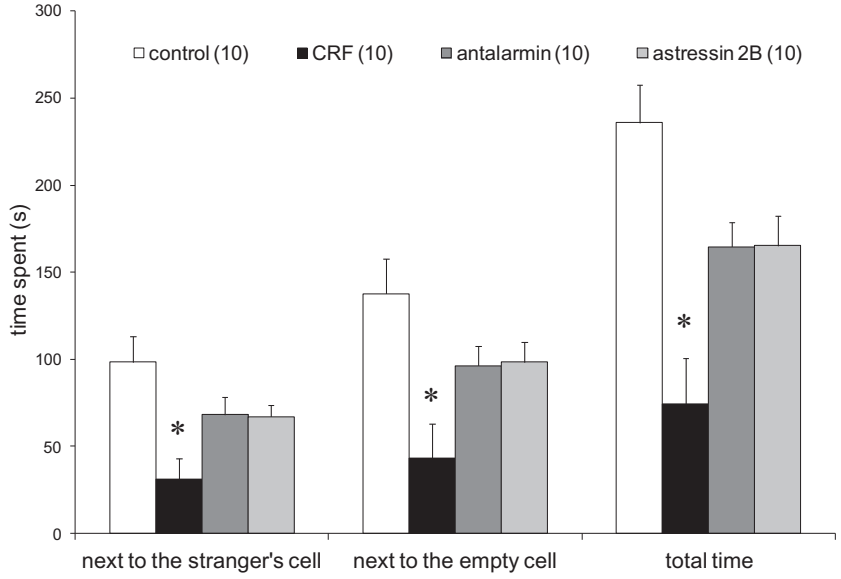

Fig. 2. The effects of CRF, antalarmin and astressin$_{2B}$ on the time of interaction in the three chamber test investigating the sociability of mice. Values are presented as means ± SEM; statistically significant difference was accepted for $p < 0.05$ and indicated with * for CRF or antalarmin or astressin$_{2B}$ *vs.* control.

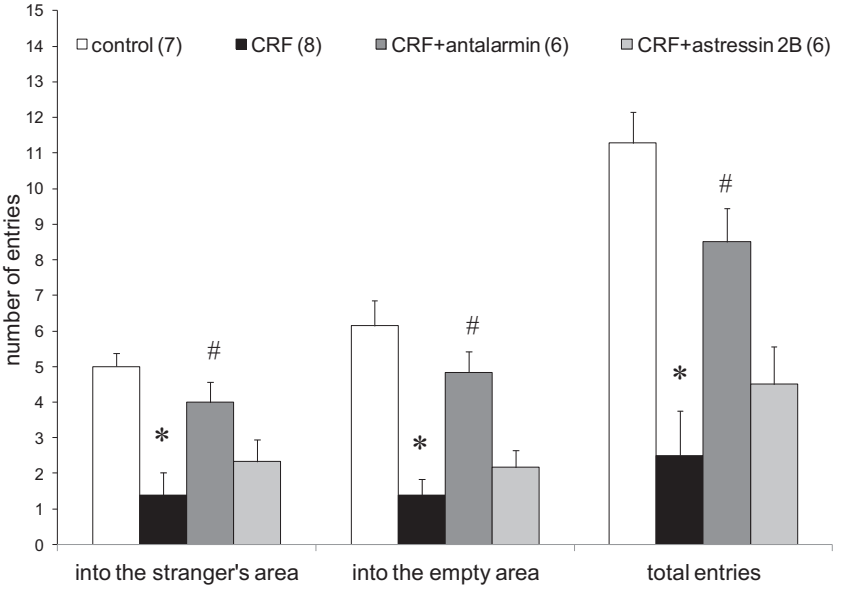

Fig. 3. The effects of CRF, CRF with antalarmin and CRF with astressin$_{2B}$ on the number of entries in the three chamber test investigating the sociability of mice. Values are presented as means ± SEM; statistically significant difference was accepted for $p < 0.05$ and indicated with * for CRF *vs.* control and # for CRF with antalarmin or astressin$_{2B}$ *vs.* CRF.

Ucn3, playing similar roles, must be also involved in social interactions. CRF decreases sociability of male mice *via* CRF_1, without reducing the locomotor activity, which is consistent with its anxiogenic effect. Ucn1 acting probably by both CRF receptors increases, while Ucn2 and Ucn3, activating selectively CRF_2, decreases the social interaction between male mice. Thus, despite of the fact that CRF_1 and CRF_2 were thought initially to play antagonistic roles in stress response, they have been found to have contrasting, but not necessarily opposite effects on social interaction.

Previous studies have already demonstrated that icv injection of CRF decreased active social interaction, without a concomitant decrease in locomotor activity, which is indicative of an anxiogenic action for CRF (Dunn and File, 1987). In addition, infusion of CRF and Ucn1 into the basolateral nucleus of the amygdala (BLA) produced anxiogenic response in male Wistar rats, reflected by a robust, dose-dependent decrease in social interaction times (Sajdyk et al., 1999). The effects of both peptides were mediated by CRF_1 (Gehlert et al., 2005; Sajdyk and Gehlert, 2000). Actually, Ucn1 was observed to be even more potent than CRF in reducing the social interaction times, which is in agreement with their relative affinities for CRF_1 (Campbell et al., 2004; Gehlert et al., 2005; Sajdyk and Gehlert, 2000). However, the effects of UCN I in rats seems to be site-specific: when injected into the BLA it produced anxiety across different endocrine and behavioral markers (Sajdyk et al., 1999); when injected into the bed nucleus of stria terminalis (BNST), it affected only the social behavior (Lee et al., 2008) and, when injected into the NACC, it did not alter any parameter of anxiety (Lee et al., 2008). In our study icv injection of CRF

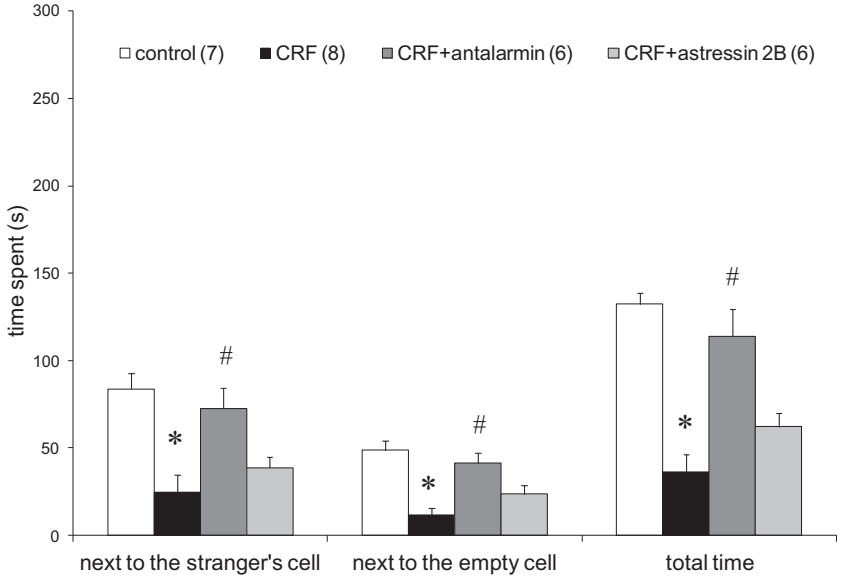

Fig. 4. The effects of CRF, CRF with antalarmin and CRF with astressin$_{2B}$ on the time of interaction in the three chamber test investigating the sociability of mice. Values are presented as means ± SEM; statistically significant difference was accepted for $p < 0.05$ and indicated with * for CRF *vs.* control and # for CRF with antalarmin or astressin$_{2B}$ *vs.* CRF.

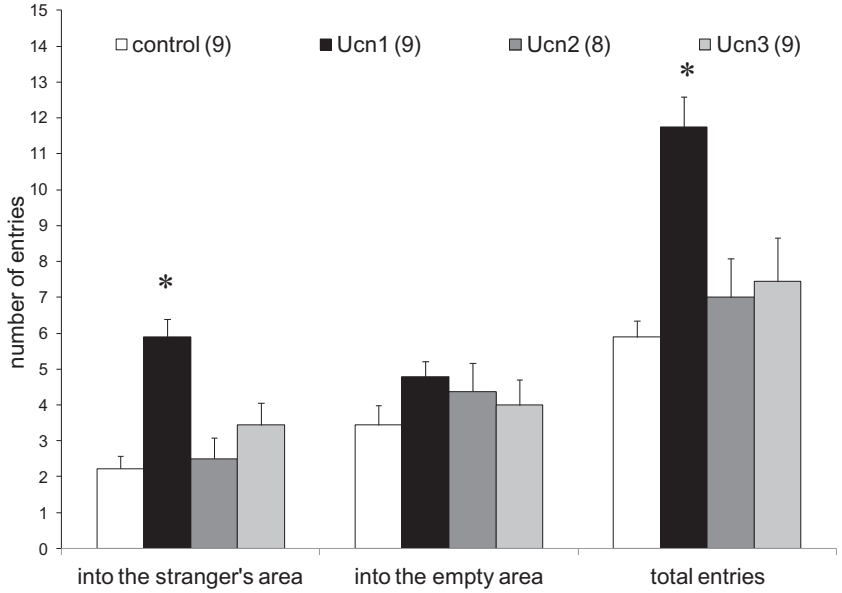

Fig. 5. The effects of Ucn1, Ucn2 and Ucn3 on the number of entries in the three chamber test investigating the sociability of mice. Values are presented as means ± SEM; statistically significant difference was accepted for $p < 0.05$ and indicated with * for Ucn *vs.* control.

decreased the social interaction of mice, without a concomitant decrease of horizontal and vertical activities, which is consistent with its anxiogenic effect that has been already indicated in rats. Although, in our knowledge, our study is the first to demonstrate that central administration CRF and Ucn1 have contrasting, anxiogenic and anxiolytic actions, respectively, on the social interaction of mice.

Previous studies also demonstrated that male, but not female Ucn2 knock-out mice exhibited more passive social interactions and reduced aggressiveness to novel conspecifics (Breu et al., 2012) and that both male and female Ucn3 and CRF_2 knock-out mice, but not Ucn2 knock-out mice, expressed an enhanced social memory and increased preference for social novelty, when compared to wild-type mice (Deussing et al., 2010). In addition, a recent study reported that mice deficient in Ucn3 or CRF_2 receptor localized specifically in the medial nucleus of the amygdala (MeA) showed decreased preference for social novelty, when compared to wild-type mice (Shemesh et al., 2016). In contrast, pharmacological activation of the CRF_2 receptors and optogenetic activation of Ucn3 neurons in the MeA proved the opposite, an increased preference for social novelty of mice (Shemesh et al., 2016). Moreover, chemogenetic inhibition of MeA Ucn3 neurons enhanced natural social behavior of the mice without affecting their hierarchal structure. The latter finding is concordant with our results indicating that icv administration of both selective agonists of CRF_2 decreases the social interaction of male CFLP mice. These studies collectively suggest that Ucn3 would modulate some aspects of social behavior *via* CRF_2 in

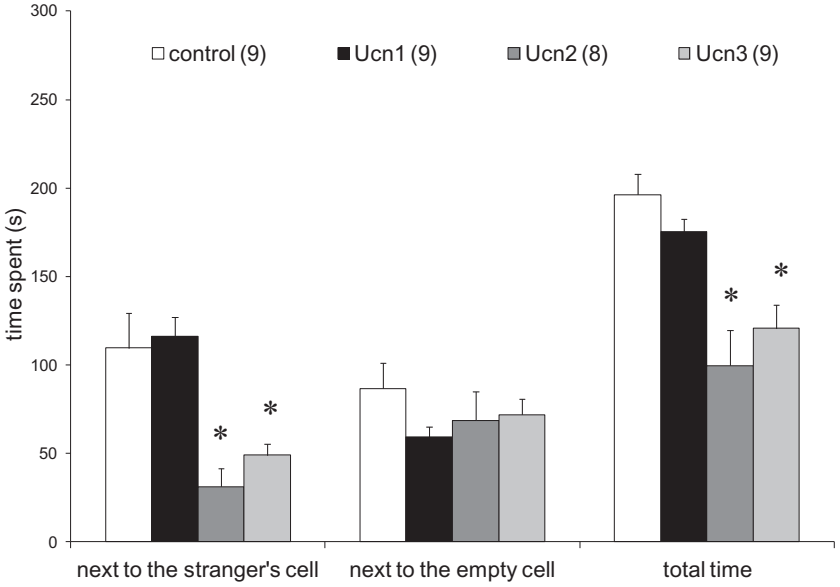

Fig. 6. The effects of Ucn1, Ucn2 and Ucn3 on the time of interaction in the three chamber test investigating the sociability of mice. Values are presented as means ± SEM; statistically significant difference was accepted for $p < 0.05$ and indicated with * for Ucn *vs.* control.

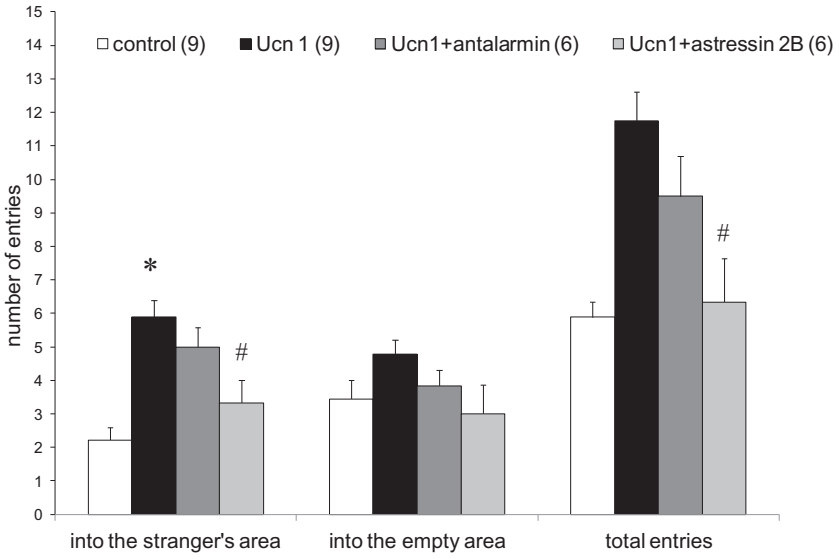

Fig. 7. The effects of Ucn1, Ucn1 with antalarmin and Ucn1 with astressin$_{2B}$ on the number of entries in the three chamber test investigating the sociability of mice. Values are presented as means ± SEM; statistically significant difference was accepted for $p < 0.05$ and indicated with * for Ucn1 *vs.* control and # for Ucn1 with antalarmin or astressin$_{2B}$ *vs.* Ucn1.

mice. However, activation of CRF_2 by icv administration of Ucn3 did not alter the social behavior in rats (Zhao et al., 2007).

In this order of thoughts, both CRF_1 and CRF_2 receptors can be considered potential targets in the therapy of diseases in which the sociability is typically altered, such as anxiety, depression, schizophrenia and autism (Crawley, 2007; Takahashi, 2001; Todorovic et al., 2005; Waters et al., 2015). However, differences between species, strains and even sexes may also exist, which makes the translation of the results from animals to humans difficult. Also, icv injection is a traditional method to describe the physiological or pharmacological effects of peptides, but certainly, this route of administration may prove limited, when it comes to therapeutical purposes. Alternative routes of administration or additional transporters should be considered when targeting CRF receptors in the human brain. Nevertheless, previous knock-out studies indicate that the role of CRF_1 and CRF_2 receptors in stress-related disorders is not a matter of simple dualism, but it depends on the different brain regions and neuron populations being activated (Janssen and Kozicz, 2013), which complicates further the interpretation of the results described following icv administration of CRF and CRF-like peptides. Previous studies performing administration of selective CRF receptor antagonists in the cerebral ventricle, the BNST and the dorsal raphe nucleus (DRN) of Syrian hamsters provided evidence that CRF_2, but not CRF_1 receptors from these regions are an important component in the neural circuitry regulating conditioned defeat (Cooper and Huhman, 2005, 2007). In comparison, our results following icv injection of selective CRF receptor antagonists alone did not provide any additional information regarding the role of these receptors in social behavior of mice. Therefore, future studies should focus on the

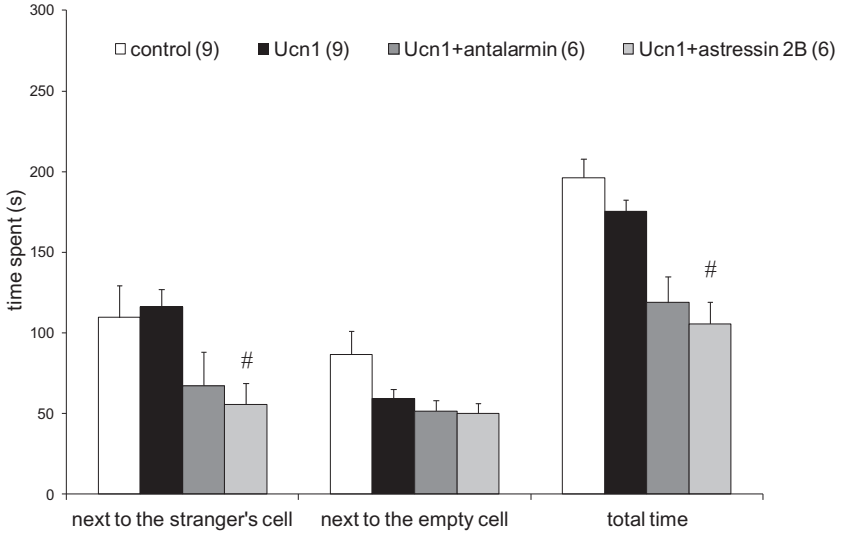

Fig. 8. The effects of Ucn1, Ucn1 with antalarmin and Ucn1 with astressin$_{2B}$ on the time of interaction in the three chamber test investigating the sociability of mice. Values are presented as means ± SEM; statistically significant difference was accepted for $p < 0.05$ and indicated with * for Ucn1 *vs.* control and # for Ucn1 with antalarmin or astressin$_{2B}$ *vs.* Ucn1.

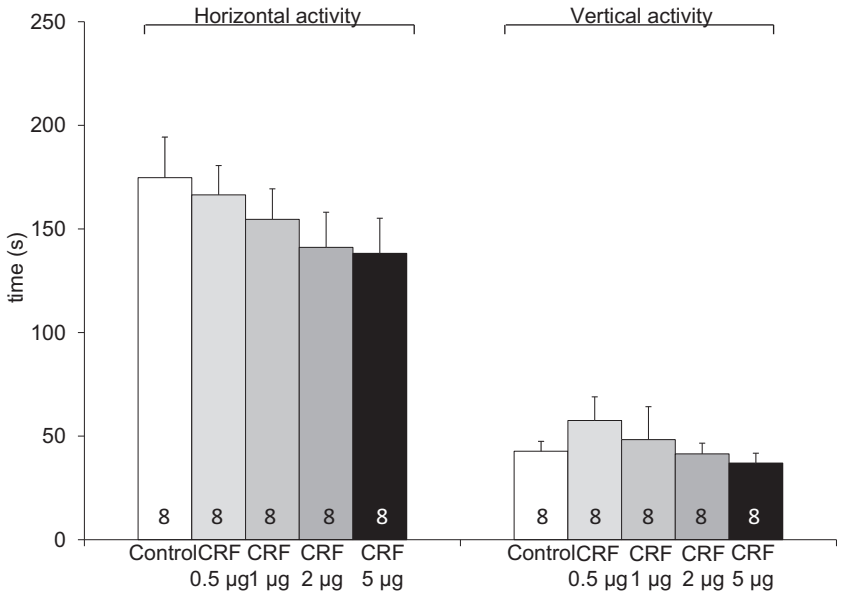

Fig. 9. The effects of CRF on the horizontal and the vertical activities of mice investigated in a conducta system. Values are presented as means ± SEM; statistically significant difference was accepted for $p < 0.05$.

administration of CRF receptor agonists and/or antagonists directly into the areas involved in both stress response and social interaction, such as the BLA, the MeA, the BNST, the NAcc etc. (Cooper and Huhman, 2005, 2007; Jasnow et al., 2004; Lim et al., 2007; Sajdyk et al., 1999; Shemesh et al., 2016).

4. Materials and methods

4.1. Animals

Male CFLP mice weighing 24–30 g were used. CFLP mice, originally called CF-1 mice, belong to an outbred strain maintained by Carworth Farms (subsequently called Anglia Laboratory Animals Limited); stock name was changed to CFLP by Lane-Petter, but this company later went out of business. In our experiment we used CFLP mice because we intended to test an outbred strain, rather than an inbred strain, such as C57/BL6 mice, which, according to previous studies, fail to exhibit preference for social novelty in the three-chamber apparatus (Pearson et al., 2010). During the experiments the mice were kept and handled in accordance with the instructions of the University of Szeged Ethical Committee for the Protection of Animals in Research. They were housed in their home cages at constant room temperature (23 °C) on a standard illumination schedule, with 12-h light and 12-h dark periods (lights on from 6:00 a.m.). Commercial food and tap water were available *ad libitum*. The mice were handled daily to minimize the effects of nonspecific stress.

4.2. Surgery

The mice were implanted with a stainless steel Luer cannula (10 mm long) aimed at the right lateral cerebral ventricle under

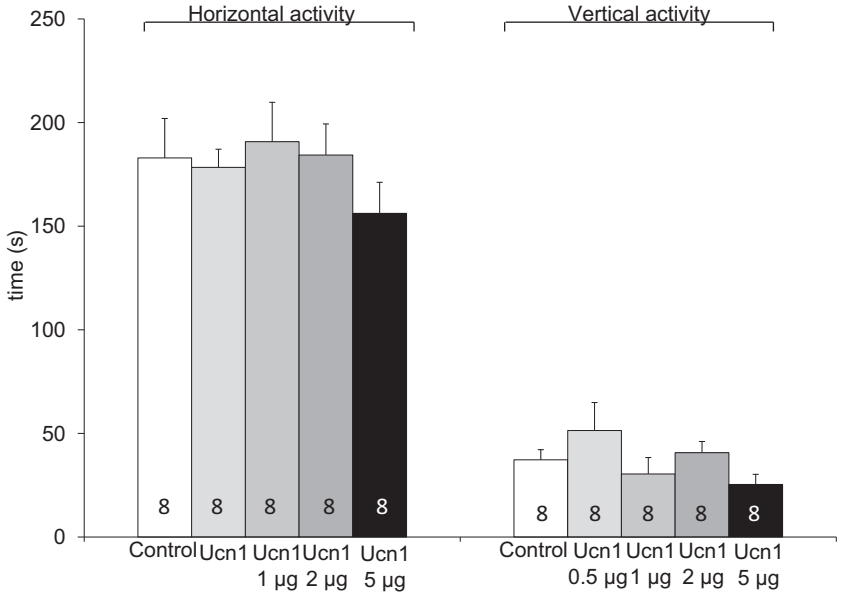

Fig. 10. The effects of Ucn1 on the horizontal and the vertical activities of mice investigated in a conducta system. Values are presented as means ± SEM; statistically significant difference was accepted for $p < 0.05$.

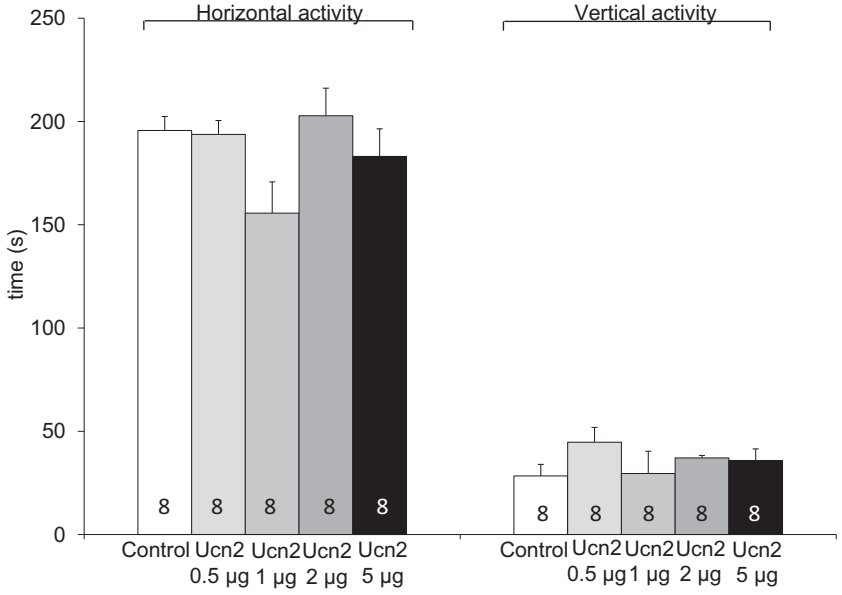

Fig. 11. The effects of Ucn2 on the horizontal and the vertical activities of mice investigated in a conducta system. Values are presented as means ± SEM; statistically significant difference was accepted for $p < 0.05$.

anesthesia with 60 mg/kg Euthanasol (CEVA-Phylaxia, Hungary). Cannulas were secured to the skull with dental cement and acrylate. The stereotaxic coordinates were 0.5 mm posterior and 0.5 mm lateral to the bregma, and 3 mm deep from the dural surface. The mice were allowed for 5 days to recover after the surgery and the permeability of the canulla was tested with methylene-blue after the experiments.

4.3. Treatment

Four experiments were performed with four different groups. In the first experiment, group 1 was treated with saline solution, group 2 with CRF, group 3 with antalarmin and group 4 with astressin$_{2B}$. In the second experiment, group 1 was treated with saline and the remaining groups were treated with CRF alone or in combination with antalarmin or astressin$_{2B}$. In the third experiment, group 1 was treated with saline and the second, the third and the fourth group with Ucn1, Ucn2 and Ucn3, respectively. In the fourth experiment, the first group was treated with saline, the remaining groups were treated with Ucn1 alone or in combination with antalarmin or astressin$_{2B}$. In case of the single administration, the injection of the agonist or the antagonist was performed 30 min before the social interaction test, whereas in case of the combined administration, injection of the antagonist was performed 30 min before that of the agonist, which means 60 min before the test itself. Saline (0.9% NaCl) solution (Biogal, Hungary) was used as vehicle for both agonist and antagonist treatment. The dose of CRF, Ucn1, Ucn2 and Ucn3 (Bachem Ltd.,

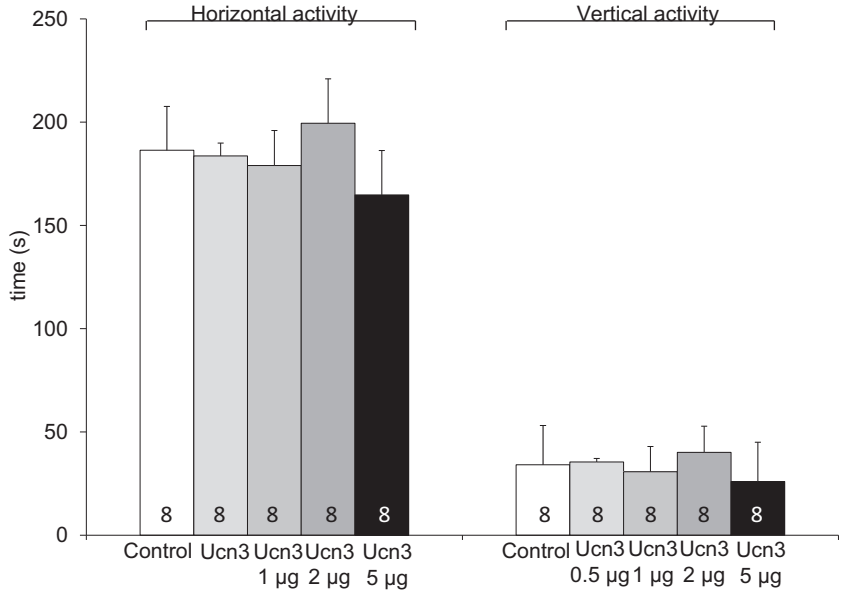

Fig. 12. The effects of Ucn3 on the horizontal and the vertical activities of mice investigated in a conducta system. Values are presented as means ± SEM; statistically significant difference was accepted for $p < 0.05$.

Switzerland) was 5 µg/2 µl, which proved effective in producing a corticosterone response in our previous experiments (Bagosi et al., 2013, 2014). The doses of antalarmin (Sigma Aldrich Inc., USA) and astressin$_{2B}$ (Sigma Aldrich Inc., USA) were 0.1 µg/2 µl and 1 µg/2 µl, which proved to abolish this response (Bagosi et al., 2014). In addition, these antagonists have been already shown to effectively block the freezing action induced by CRF, without altering the baseline social interaction (Bhutada et al., 2010; Zorrilla et al., 2013).

4.4. Social interaction test

Thirty minutes after ICV injection of the peptides mice were investigated in a social interaction test arena invented by Crawley and colleagues (Crawley, 2007). The apparatus was a rectangular, three-chamber box. Each chamber of 19 × 45 × 25 cm and were made from clear Plexiglas, with an open middle section, which allowed free access to each chamber. The right and left chambers could be isolated from the middle one by using two dividing Plexiglass walls. Two identical, wire cup-like cage of 10 × 17 cm with removable lids that large enough to hold a single mouse were placed vertically inside the apparatus, one in each side chamber. Each cage was comprised of metal wires to allow for air exchange between the interior and exterior of the cylinder but small enough to prevent direct physical interactions between an animal on the inside with one on the outside. In the our experiments an unknown mouse (a male set in a cage) was put in the first chamber and an unknown object (an empty cage) was put in the opposite chamber. First the tested mouse was habituated with the middle chamber for 5 min and then allowed to explore the remaining chambers for another 5 min, during which the number of entries and the time of interaction were measured. The mouse was considered to be in the chamber when its head and four paws have entered into the chamber. The time of interaction was represented by the time spent in an area 3–5 cm around the cage with stretching, grooming and licking of the body of the other mouse or the grids. Behavioral testing were performed between 9:00 a.m. and 13:00 p.m. General room lighting was 650 lux. The person who made the observation was at 2 meters away from the apparatus. After each trial, all chambers were cleaned with 70% ethanol and then with Clidox 1:5:1 to prevent olfactory cue bias and to ensure proper disinfection, respectively (Kaidanovich-Beilin et al., 2011).

4.5. Open-field test

In parallel with the social interaction test, the horizontal locomotor activity and the vertical locomotor activity of the mice were monitored in an *in vivo* conducta system, based on the principles of an open-field test (Conducta 1.0, Experimetria Ltd., Budapest, Hungary). The conducta apparatus was a square open-field black cage with a side length of 60 cm, surrounded by a 40 cm high wall. The floor of the cage was divided in 36 (6 × 6) small squares. Five by five rows of photocell beams allowed a computer-based system to register the behavioral activity of each animal. A 60 W light was situated 1 m above the arena floor. Each tested mouse was placed in the center of the open field for 5 min for habituation and then monitored for 30 min during free exploration. The box was cleaned between sessions with 96% ethyl-alcohol (Reanal Ltd., Hungary).

4.6. Statistical analysis

Statistical analysis of the results was performed by analysis of variance (ANOVA, GraphPad Prism Software). The differences between groups were tested by one-way ANOVA followed by Tukey *post-hoc* comparison test for the social interaction test or Dunnett *post-hoc* comparison test for the open-field test. A probability level of 0.05 or less was accepted as indicating a statistically significant difference.

Acknowledgement

This study was supported by the Neuroscience Research Group of the Hungarian Academy of Sciences and the Hungarian Brain Research Program.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.brainres.2017.03.003.

References

Backstrom, T., Winberg, S., 2013. Central corticotropin releasing factor and social stress. Front. Neurosci. 7, 117.

Bagosi, Z., Csabafi, K., Palotai, M., Jaszberenyi, M., Foldesi, I., Gardi, J., Szabo, G., Telegdy, G., 2013. The interaction of Urocortin II and Urocortin III with amygdalar and hypothalamic cotricotropin-releasing factor (CRF)–reflections on the regulation of the hypothalamic-pituitary-adrenal (HPA) axis. Neuropeptides 47, 333–338.

Bagosi, Z., Csabafi, K., Palotai, M., Jaszberenyi, M., Foldesi, I., Gardi, J., Szabo, G., Telegdy, G., 2014. The effect of urocortin I on the hypothalamic ACTH secretagogues and its impact on the hypothalamic-pituitary-adrenal axis. Neuropeptides 48, 15–20.

Behan, D.P., De Souza, E.B., Lowry, P.J., Potter, E., Sawchenko, P., Vale, W.W., 1995. Corticotropin releasing factor (CRF) binding protein: a novel regulator of CRF and related peptides. Front. Neuroendocrinol. 16, 362–382.

Bhutada, P., Mundhada, Y., Bansod, K., Ubgade, A., Quazi, M., Umathe, S., Mundhada, D., 2010. Reversal by quercetin of corticotrophin releasing factor induced anxiety- and depression-like effect in mice. Prog. Neuropsychopharmacol. Biol. Psychiatry 34, 955–960.

Breu, J., Touma, C., Holter, S.M., Knapman, A., Wurst, W., Deussing, J.M., 2012. Urocortin 2 modulates aspects of social behaviour in mice. Behav. Brain Res. 233, 331–336.

Campbell, B.M., Morrison, J.L., Walker, E.L., Merchant, K.M., 2004. Differential regulation of behavioral, genomic, and neuroendocrine responses by CRF infusions in rats. Pharmacol. Biochem. Behav. 77, 447–455.

Carpenter, R.E., Korzan, W.J., Bockholt, C., Watt, M.J., Forster, G.L., Renner, K.J., Summers, C.H., 2009. Corticotropin releasing factor influences aggression and monoamines: modulation of attacks and retreats. Neuroscience 158, 412–425.

Carpenter, R.E., Maruska, K.P., Becker, L., Fernald, R.D., 2014. Social opportunity rapidly regulates expression of CRF and CRF receptors in the brain during social ascent of a teleost fish, Astatotilapia burtoni. PLoS One 9, e96632.

Carrasco, G.A., Van de Kar, L.D., 2003. Neuroendocrine pharmacology of stress. Eur. J. Pharmacol. 463, 235–272.

Chang, C.P., Pearse 2nd, R.V., O'Connell, S., Rosenfeld, M.G., 1993. Identification of a seven transmembrane helix receptor for corticotropin-releasing factor and sauvagine in mammalian brain. Neuron 11, 1187–1195.

Cooper, M.A., Huhman, K.L., 2005. Corticotropin-releasing factor type II (CRF-sub-2) receptors in the bed nucleus of the stria terminalis modulate conditioned defeat in Syrian hamsters (Mesocricetus auratus). Behav. Neurosci. 119, 1042–1051.

Cooper, M.A., Huhman, K.L., 2007. Corticotropin-releasing factor receptors in the dorsal raphe nucleus modulate social behavior in Syrian hamsters. Psychopharmacology 194, 297–307.

Crawley, J.N., 2007. What's Wrong With My Mouse?: Behavioral Phenotyping of Transgenic and Knockout Mice. Wiley-Interscience, Hoboken, N.J..

Dautzenberg, F.M., Hauger, R.L., 2002. The CRF peptide family and their receptors: yet more partners discovered. Trends Pharmacol. Sci. 23, 71–77.

Deussing, J.M., Breu, J., Kuhne, C., Kallnik, M., Bunck, M., Glasl, L., Yen, Y.C., Schmidt, M.V., Zurmuhlen, R., Vogl, A.M., Gailus-Durner, V., Fuchs, H., Holter, S.M., Wotjak, C.T., Landgraf, R., de Angelis, M.H., Holsboer, F., Wurst, W., 2010. Urocortin 3 modulates social discrimination abilities via corticotropin-releasing hormone receptor type 2. J. Neurosci. 30, 9103–9116.

Dunn, A.J., File, S.E., 1987. Corticotropin-releasing factor has an anxiogenic action in the social interaction test. Horm. Behav. 21, 193–202.

Fekete, E.M., Zorrilla, E.P., 2007. Physiology, pharmacology, and therapeutic relevance of urocortins in mammals: ancient CRF paralogs. Front. Neuroendocrinol. 28, 1–27.

Gehlert, D.R., Shekhar, A., Morin, S.M., Hipskind, P.A., Zink, C., Gackenheimer, S.L., Shaw, J., Fitz, S.D., Sajdyk, T.J., 2005. Stress and central Urocortin increase anxiety-like behavior in the social interaction test via the CRF1 receptor. Eur. J. Pharmacol. 509, 145–153.

Grammatopoulos, D.K., Randeva, H.S., Levine, M.A., Kanellopoulou, K.A., Hillhouse, E.W., 2001. Rat cerebral cortex corticotropin-releasing hormone receptors: evidence for receptor coupling to multiple G-proteins. J. Neurochem. 76, 509–519.

Hostetler, C.M., Ryabinin, A.E., 2013. The CRF system and social behavior: a review. Front. Neurosci. 7, 92.

Jamieson, P.M., Li, C., Kukura, C., Vaughan, J., Vale, W., 2006. Urocortin 3 modulates the neuroendocrine stress response and is regulated in rat amygdala and hypothalamus by stress and glucocorticoids. Endocrinology 147, 4578–4588.

Janssen, D., Kozicz, T., 2013. Is it really a matter of simple dualism? Corticotropin-releasing factor receptors in body and mental health. Front. Endocrinol. (Lausanne) 4, 28.

Jasnow, A.M., Davis, M., Huhman, K.L., 2004. Involvement of central amygdalar and bed nucleus of the stria terminalis corticotropin-releasing factor in behavioral responses to social defeat. Behav. Neurosci. 118, 1052–1061.

Kaidanovich-Beilin, O., Lipina, T., Vukobradovic, I., Roder, J., Woodgett, J.R., 2011. Assessment of social interaction behaviors. J. Vis. Exp.

Lee, Y., Fitz, S., Johnson, P.L., Shekhar, A., 2008. Repeated stimulation of CRF receptors in the BNST of rats selectively induces social but not panic-like anxiety. Neuropsychopharmacology 33, 2586–2594.

Lewis, K., Li, C., Perrin, M.H., Blount, A., Kunitake, K., Donaldson, C., Vaughan, J., Reyes, T.M., Gulyas, J., Fischer, W., Bilezikjian, L., Rivier, J., Sawchenko, P.E., Vale, W.W., 2001. Identification of urocortin III, an additional member of the corticotropin-releasing factor (CRF) family with high affinity for the CRF2 receptor. Proc. Natl. Acad. Sci. U.S.A. 98, 7570–7575.

Lim, M.M., Liu, Y., Ryabinin, A.E., Bai, Y., Wang, Z., Young, L.J., 2007. CRF receptors in the nucleus accumbens modulate partner preference in prairie voles. Horm. Behav. 51, 508–515.

Martinez, V., Wang, L., Rivier, J.E., Vale, W., Tache, Y., 2002. Differential actions of peripheral corticotropin-releasing factor (CRF), urocortin II, and urocortin III on gastric emptying and colonic transit in mice: role of CRF receptor subtypes 1 and 2. J. Pharmacol. Exp. Ther. 301, 611–617.

Maruyama, H., Makino, S., Noguchi, T., Nishioka, T., Hashimoto, K., 2007. Central type 2 corticotropin-releasing hormone receptor mediates hypothalamic-pituitary-adrenocortical axis activation in the rat. Neuroendocrinology 86, 1–16.

Pearson, B.L., Defensor, E.B., Blanchard, D.C., Blanchard, R.J., 2010. C57BL/6J mice fail to exhibit preference for social novelty in the three-chamber apparatus. Behav. Brain Res. 213, 189–194.

Pelleymounter, M.A., Joppa, M., Ling, N., Foster, A.C., 2004. Behavioral and neuroendocrine effects of the selective CRF2 receptor agonists urocortin II and urocortin III. Peptides 25, 659–666.

Reyes, T.M., Lewis, K., Perrin, M.H., Kunitake, K.S., Vaughan, J., Arias, C.A., Hogenesch, J.B., Gulyas, J., Rivier, J., Vale, W.W., Sawchenko, P.E., 2001. Urocortin II: a member of the corticotropin-releasing factor (CRF) neuropeptide family that is selectively bound by type 2 CRF receptors. Proc. Natl. Acad. Sci. U.S.A. 98, 2843–2848.

Richard, D., Lin, Q., Timofeeva, E., 2002. The corticotropin-releasing factor family of peptides and CRF receptors: their roles in the regulation of energy balance. Eur. J. Pharmacol. 440, 189–197.

Sajdyk, T.J., Schober, D.A., Gehlert, D.R., Shekhar, A., 1999. Role of corticotropin-releasing factor and urocortin within the basolateral amygdala of rats in anxiety and panic responses. Behav. Brain Res. 100, 207–215.

Sajdyk, T.J., Gehlert, D.R., 2000. Astressin, a corticotropin releasing factor antagonist, reverses the anxiogenic effects of urocortin when administered into the basolateral amygdala. Brain Res. 877, 226–234.

Shemesh, Y., Forkosh, O., Mahn, M., Anpilov, S., Sztainberg, Y., Manashirov, S., Shlapobersky, T., Elliott, E., Tabouy, L., Ezra, G., Adler, E.S., Ben-Efraim, Y.J., Gil, S., Kuperman, Y., Haramati, S., Dine, J., Eder, M., Deussing, J.M., Schneidman, E., Yizhar, O., Chen, A., 2016. Ucn3 and CRF-R2 in the medial amygdala regulate complex social dynamics. Nat. Neurosci. 19, 1489–1496.

Skelton, K.H., Owens, M.J., Nemeroff, C.B., 2000. The neurobiology of urocortin. Regul. Pept. 93, 85–92.

Suda, T., Kageyama, K., Sakihara, S., Nigawara, T., 2004. Physiological roles of urocortins, human homologues of fish urotensin I, and their receptors. Peptides 25, 1689–1701.

Takahashi, K., Totsune, K., Murakami, O., Shibahara, S., 2004. Urocortins as cardiovascular peptides. Peptides 25, 1723–1731.

Takahashi, L.K., 2001. Role of CRF(1) and CRF(2) receptors in fear and anxiety. Neurosci. Biobehav. Rev. 25, 627–636.

Todorovic, C., Jahn, O., Tezval, H., Hippel, C., Spiess, J., 2005. The role of CRF receptors in anxiety and depression: implications of the novel CRF1 agonist cortagine. Neurosci. Biobehav. Rev. 29, 1323–1333.

Vale, W., Spiess, J., Rivier, C., Rivier, J., 1981. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. Science 213, 1394–1397.

Vaughan, J., Donaldson, C., Bittencourt, J., Perrin, M.H., Lewis, K., Sutton, S., Chan, R., Turnbull, A.V., Lovejoy, D., Rivier, C., et al., 1995. Urocortin, a mammalian neuropeptide related to fish urotensin I and to corticotropin-releasing factor. Nature 378, 287–292.

Waters, R.P., Rivalan, M., Bangasser, D.A., Deussing, J.M., Ising, M., Wood, S.K., Holsboer, F., Summers, C.H., 2015. Evidence for the role of corticotropin-releasing factor in major depressive disorder. Neurosci. Biobehav. Rev. 58, 63–78.

Zhao, Y., Valdez, G.R., Fekete, E.M., Rivier, J.E., Vale, W.W., Rice, K.C., Weiss, F., Zorrilla, E.P., 2007. Subtype-selective corticotropin-releasing factor receptor agonists exert contrasting, but not opposite, effects on anxiety-related behavior in rats. J. Pharmacol. Exp. Ther. 323, 846–854.

Zorrilla, E.P., Roberts, A.J., Rivier, J.E., Koob, G.F., 2013. Anxiolytic-like effects of antisauvagine-30 in mice are not mediated by CRF2 receptors. PLoS ONE 8, e63942.