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ORIGINAL ARTICLE

Urocortin 1 administered into the hypothalamic supraoptic nucleus affects open-field behaviour in rats

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Abstract The presence of both Urocortin 1 (Ucn1) and corticotropin-releasing factor 2 receptors (CRF_2R) in the hypothalamic supraoptic nucleus (SON) suggests that endogenous Ucn1 released within this brain area acts as a local signal that might be involved in the regulation of not only endocrine but also behavioural stress responses. To test this hypothesis, we monitored the effects induced by the administration of a range of doses of synthetic Ucn1 (0.001–1.0 μg) bilaterally into the SON of rats in the open field test (OFT). Ucn1 administration produced an inverted U-shaped dose–response curve on OFT behaviour, in particular the dose of 0.01 μg of Ucn1 significantly increased the number of rearing and grooming episodes without affecting locomotion. In addition, this dosage augmented also the latency to visit the centre of the open field. Pretreatment with the CRF_2R antagonist, astressin-2B (0.1 μg) normalized Ucn1 treatment-induced effects. These results suggest that Ucn1 released within the SON area interacts with CRF_2R to control the state of arousal.

Keywords Urocortin 1 · Corticotropin-releasing factor 2 receptor · Astressin-2B · Anxiety-like behaviour · Grooming behaviour

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Introduction

The neuropeptide family of the corticotropin-releasing factor (CRF), both ligands and receptors, have been implicated in the regulation of endocrine and behavioural stress response (Bale and Vale 2004; Keck 2006; Landgraf 2006). There is convincing pre-clinical and clinical evidence that signalling via the CRF_1 receptor (CRF_1R) is involved in the development of psychiatric illnesses (Arzt and Holsboer 2006). However, the role played by CRF_2R in this context remains unclear. Some studies report that the activation of CRF_2R reduces anxiety (Bale et al. 2000; Valdez et al. 2003) while others describe opposite effects (Bakshi et al. 2002; Risbrough et al. 2004). Recently, Henry et al. (2006) proposed that the amount of septal CRF_2R activation on anxiety is likely to depend upon the stressor intensity.

Urocortin 1 (Ucn1), a 40-amino acid peptide, is a member of the CRF peptide family. It binds to both receptors, CRF_1R and CRF_2R, with a higher affinity than CRF itself. The overlapping distribution of Ucn1 and CRF_2R expressing neurons suggests that Ucn1 may serve in addition to CRF as an endogenous CRF_2R ligand (Hauger et al. 2003). Indeed, Ucn1 administered intracerebroventricularly has been reported to produce anxiety-like behaviour and long-lasting anorectic effects in rats (Spina et al. 1996, 2002). Within the rodent brain, Ucn1 mRNA and the peptide have been detected in the non-preganglionic Edinger–Westphal nucleus, lateral superior olivary and hypothalamus (Bittencourt et al. 1999; Kozicz et al. 1998; Vaughan et al. 1995). Although, it has been reported that within the hypothalamus Ucn1 is synthesized predominantly in magnocellular neurons of the supraoptic nucleus (SON; Spina et al. 2004), little information is available about the consequences of Ucn1 signalling in this

brain area (Spina et al. 2004). This, however, would be of particular interest with respect to the fact that the magnocellular neurons of the SON are able to release neuropeptides such as vasopressin and oxytocin from the axons and somata into the extracellular fluid under stress conditions independently upon peripheral secretion (Engelmann et al. 2001, 2006). After this non-synaptic intranuclear release, the neuropeptides are likely to act as neuromodulators by binding to specific binding sites within the SON and its close (and also remote) vicinity to contribute to endocrine and behavioural regulation (Engelmann et al. 1994; Landgraf and Neumann 2004; Ludwig and Leng 2006; Wotjak et al. 2002). In fact, the presence of mRNA coding for CRF_2R (Van Pett et al. 2000) in the SON area implies also that urocortin acts as a local neuromodulator.

The present study was designed to investigate whether bilateral administration of synthetic Ucn1 into the SON of rats affects parameters in the open field test (OFT), which have been suggested to reflect anxiety-like behaviour. To further characterize the specific receptor (sub)type via which synthetic Ucn1 may act, animals were pre-treated with a selective CRF_2R antagonist (astressin-2B; Ast) and the behavioural consequences monitored.

Methods

Animals

Eighty-two male Wistar rats (Harlan-Winkelmann, Germany), weighing 200 ± 20 g at the start of the experiments, were housed two per cage under controlled laboratory conditions and maintained at a 12:12 h reversed light–dark schedule (lights on at 6 p.m.). Between six and ten rats were allocated to a specific treatment per group. Animals had free access to standard rat food and tap water. They were allowed a 1-week period of acclimation to the animal room and handled three times a week before surgery.

All behavioural tests were performed during the dark phase of the day–night-cycle. All procedures were conducted in compliance with the EEC recommendations for the care and use of laboratory animals (86/609/CEE).

Surgery

Rats were anaesthetized with a mixture of Xylazine (4 mg/kg; Rompun®, Bayer, Germany) and Ketamine (50 mg/kg; Ketavet®, Pharmacia Upjohn, Germany), administered intraperitoneally and placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA). Stainless steel guide cannulas (23 gauge) were implanted bilaterally just above the SON according to the atlas of Paxinos and Watson [(1998); −0.8 mm anterior to bregma, +1.7 mm lateral from mid line, −7.6 ventral from skull surface; flat skull]. The cannulas were secured to the skull using stainless steel screws and dental cement and closed with a removable stylet. Animals were allowed a 1-week recovery period before testing.

Drugs

Ucn1 and Ast (kindly provided by the Clayton foundation Laboratories for peptide Biology, The Salk Institute, La Jolla, CA, USA) were initially dissolved in double-distilled water (pH 6.7) and diluted to final concentrations with sterile saline. Solutions were freshly prepared just before the start of each experiment, and kept on ice.

Drug administration and histology

Intra-SON injection was performed in freely moving animals. Peptides were infused through 33 gauge injector cannulas, which were 12.7 mm long, attached to Hamilton microsyringes mounted on an automated pump. Peptides were infused over a 2-min-period with a volume of 2 µl per side. Injectors were kept in place for another 1 min to prevent back flow leakage. The injectors were then removed and replaced by stylets. Upon completion of the experiment, rats received a terminal anaesthesia of isoflurane, the brains were removed and rapidly frozen at −80°C. Brains were sectioned and every fourth section (25 µm) was processed for histological assessment of placement of cannula using a standard protocol for cresylviolet staining. Only data of animals in which the cannula placement was found correctly located in the SON (Fig. 1) were included in the statistical analysis.

For the dose–response experiments, animals were infused with either vehicle or one of the following Ucn1

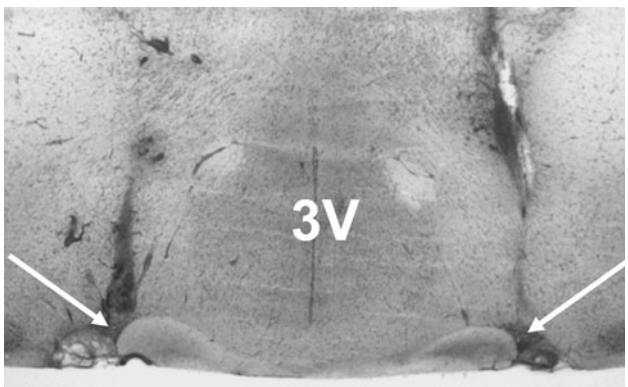

Fig. 1 Verification of SON injection sites. Representative microphotograph of a cresylviolet stained rat brain slice showing the tracks caused by the bilateral intra-SON injection. *White arrows* indicate the injection side within the SON. *3V* third ventricle

doses: 0.001, 0.01, 0.1, 0.5, 1 μg. The second experiment consisted of four groups: saline + saline, saline + Ucn1, Ast + saline, Ast + Ucn1. Each rat was pre-treated with either saline or Ast (0.1 μg), 15 min prior to the second injection of either saline or Ucn1 (0.01 μg).

Open field test

The OFT apparatus was made out of polyacrylics and consisted of a white square floor (100 × 100 cm, also divided in 20 × 20 cm squares by black lines) and of 40 cm high walls. Habituation and testing were done under the same conditions of uniform bright illumination (250 ± 10 lux) and a white noise of 60 db. Rats were habituated for 10 min to the open field for two consecutive days prior to the testing day. Habituation was performed to establish stable behavioural baseline levels and to minimize the individual variations in the anxiety-like behaviour due to novelty in all animals. On the testing day, the rat was placed in the centre of the field 60 min after the Ucn1 or saline administration. The 60 min timepoint was chosen on the basis of our previous data, which shows that Ucn1 affects behaviour within 30–90 min of intra-SON administration (Fatima et al. 2006). The behaviour was recorded for 10 min using a videorecorder connected to a video camera located above the centre of the arena. The tapes were later analysed by a trained observer, unaware of the animal's treatment, by pressing pre-defined keys on a PC using the Eventlog software (v.1.0, Robert Hendersen, 1986). The following behaviours were scored: number of rearing episodes (erected posture sustained by the hind paws on the floor), number of lines crossed and latency to visit the centre, time and number of episodes, and latency to groom (including washing or mouthing of forelimbs, hind paws, face, body and genital). For a detailed analysis of grooming elements, grooming of head, face, and forelimbs were defined as "head washing" whereas grooming of hind paws, body, genitals and tail were defined as "body grooming". After each testing, the open field was carefully wiped with a damp sponge and dried.

Data analysis

Statistical analysis was performed using the SYSTAT software (v. 7.0 SPSS Inc., 1997). A preliminary analysis of the untransformed behavioural data revealed significant difference in the variances of the treatment groups. This was further confirmed by testing for equality of variance using Bartlett's test. To correct for this heteroscedasticity, we introduced weights to each observation and used weighted one-way ANOVA to assess differences between and within the treatment groups (Kulinskayaa and Dollingerb 2007). All values are expressed as weighted mean values ± SEM. Post hoc analysis was performed using Fishers LSD-test. A P value less than 0.05 was considered significant whereas a value of P higher than 0.05 but less than 0.06 was considered as tendency to achieve significant differences (Rosener 2006).

Results

Dose–response curve

Administration of Ucn1 directly into the SON produced an inverted U-shaped dose–response curve on the behavioural parameters measured in the OFT. The results of F-statistics obtained after performing weighted one-way ANOVA are complied in Table 1. A significant increase in the number of rearing episodes was observed at the doses of 0.001 and 0.01 μg of Ucn1 ($P = 0.01$). There was also a tendency towards an increased number of grooming episodes in the group receiving 0.01 μg Ucn1 ($P = 0.06$). As shown in Fig. 2, the maximum number of line crossings (104.59 ± 20.06), rearing (29.32 ± 3.72) and grooming episodes (9.25 ± 1.75) were observed at a dose of 0.01 μg of Ucn1. These effects vanished at concentrations higher than 0.01 μg. At the highest concentration (1 μg), the number of line crossings, rearing and grooming episodes were significantly reduced ($P = 0.04$, $P < 0.01$ and $P = 0.019$, respectively) compared to 0.01 μg Ucn1. In general, the most pronounced behavioural effects were observed at the dose of 0.01 μg Ucn1. Therefore, this dose was selected for further testing the effects of Ucn1 in presence of Ast.

Table 1 Results of F-statistics obtained after one-way ANOVA

	F ratio	P value
Dose response of Ucn1[a]		
Rearing episodes	4.08	0.01
Grooming episodes	2.37	0.05
Line crossing	2.06	0.08
Effects of Ucn1 in presence of Ast[b]		
Latency to visit centre	3.65	0.03
Grooming episodes	4.74	0.01
Total time spent in grooming	4.11	0.02
Head washing episodes	4.01	0.01
Time spent in head washing	3.61	0.02
Body grooming episodes	5.91	0.01
Time Spent in body grooming	3.29	0.03

[a] Degrees of freedom = 5,49

[b] Degrees of freedom = 3,31

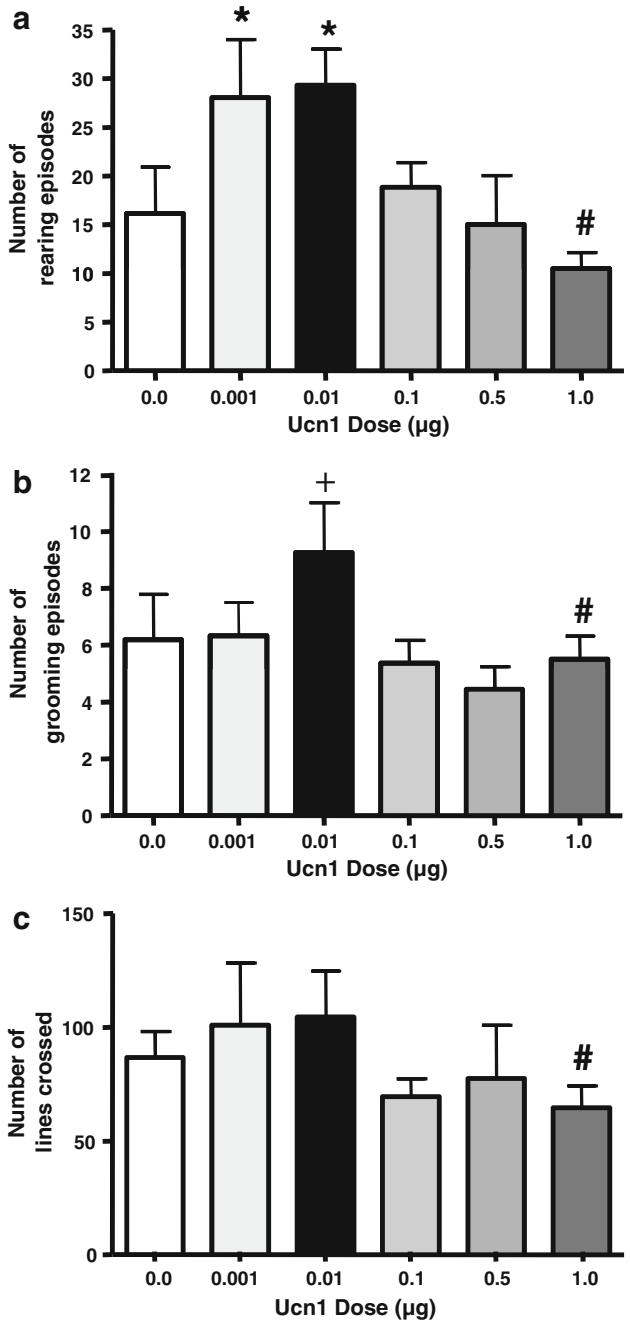

Fig. 2 Dose–response curve of intra-SON administered Ucn1 for rearing (**a**), grooming (**b**), and line crossing (**c**) episodes in rats in the OFT. Weighted mean ± SEM. Ucn1 administered bilaterally into SON produced an inverted U-shaped dose–response curve having a maximal response at dose of 0.01 μg beyond which the higher doses were unable to produce an effect. * $P < 0.05$ compared to saline; # $P < 0.05$ compared to 0.01 μg Ucn1; + $0.05 < P < 0.06$ compared to saline. $n = 6$–10 per treatment group

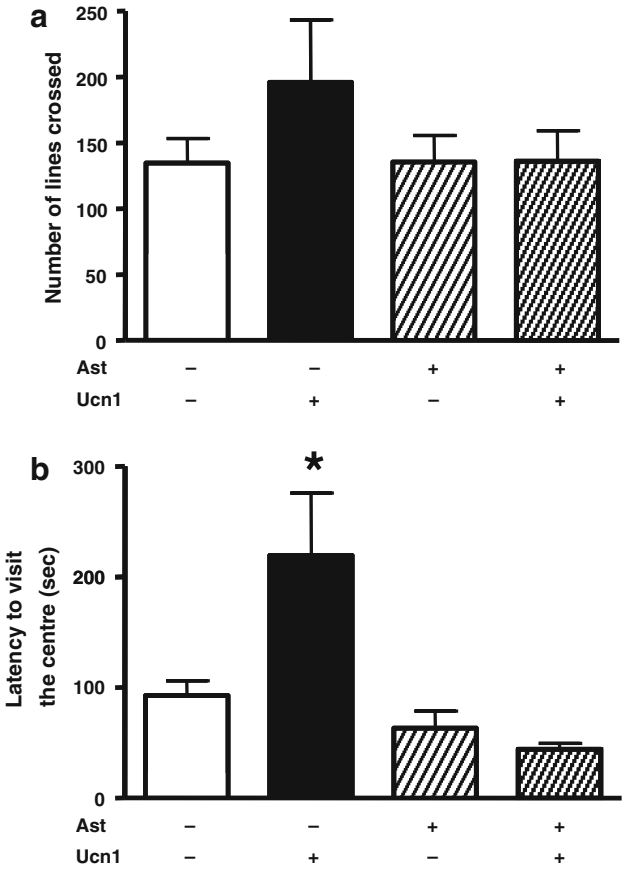

Fig. 3 Effect of Intra-SON Ucn1 administration in the presence of CRF_2R antagonist Ast, on locomotion in terms on number of lines crossed (**a**), and latency to visit the centre (**b**) of the open field. Weighted mean ± SEM. **a** None of the treatments affected significantly the locomotor activity in the OFT significantly. **b** Ucn1 significantly increased the latency to visit the centre. This effect was blocked by Ast pre-treatment. * $P < 0.05$ compared to saline and Ast pre-treated group. $n = 8$ per treatment group

Effects of Ucn1 in the presence of a selective CRF_2R antagonist

A one-way ANOVA revealed that there was a significant treatment effect on different behavioural parameters in the OFT. As illustrated in Fig. 3a, neither Ucn1 nor Ast infusion caused a significant effect on locomotion, as measured by the number of lines crossed. However, Ucn1 significantly increased the latency to visit the centre ($P = 0.01$; Fig. 3b). This effect was blocked in the Ast pre-treated group ($P = 0.04$).

The results of grooming behaviour are presented in Fig. 4. Post hoc testing revealed in animals treated with 0.01 μg of Ucn1 a significant increase in both number of grooming episodes ($P = 0.01$) and total time spent grooming ($P = 0.01$). Pre-treatment with Ast significantly reduced time spent grooming ($P < 0.01$), latency to groom ($P = 0.02$), and number of grooming episodes ($P = 0.02$). Interestingly, treatment with Ast per se also significantly increased the number of grooming episodes ($P = 0.01$; Fig. 4c). However, a detailed analysis of distinct elements of the grooming response elicited by Ucn1 and Ast revealed that the two peptides had differential effects

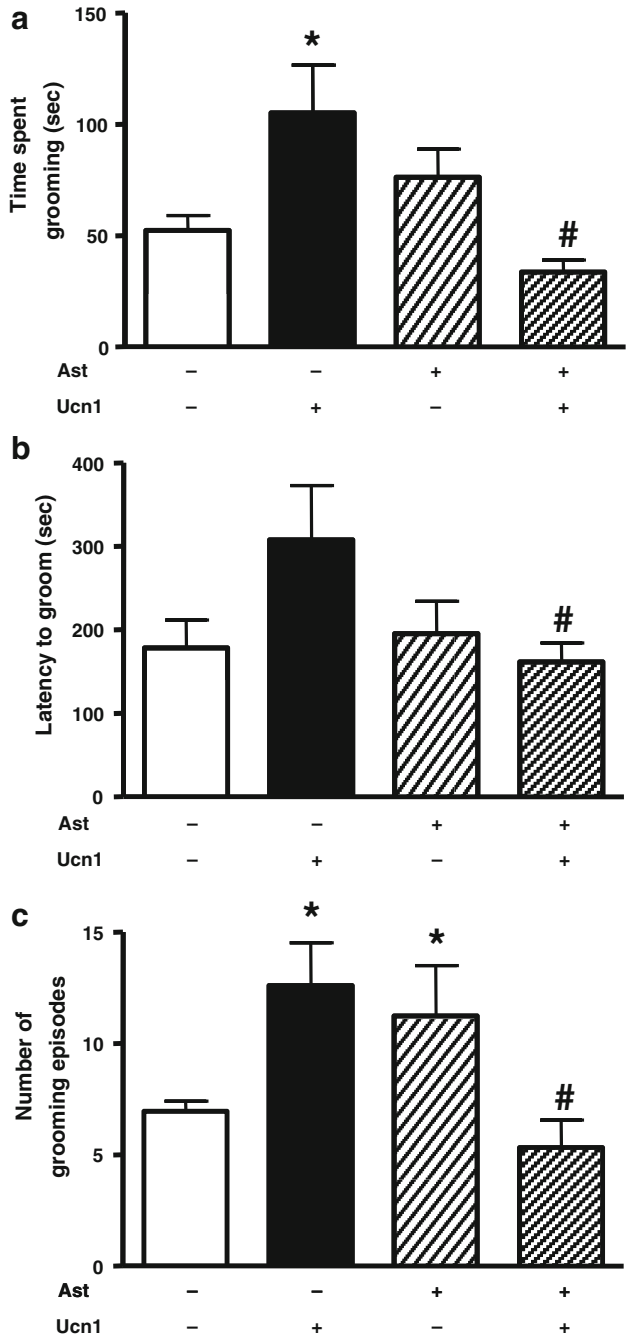

Fig. 4 Effects of Intra-SON Ucn1 administration in presence of CRF_2R antagonist Ast, on the time spent grooming (**a**), number of grooming episodes (**b**) and latency to groom (**c**) in the OFT. Weighted mean ± SEM. Ast pre-treatment significantly decreased Ucn1 induced grooming in animals. * $P < 0.05$ compared to saline; # $P < 0.05$ compared to 0.01 µg Ucn1. $n = 8$ per treatment group

(Table 2a, b). Ucn1 treatment (0.01 µg) significantly increased both number of episodes ($P = 0.04$) and time spent in head washing ($P = 0.04$). In addition, it also increased the total time spent in body grooming ($P = 0.02$) but failed to affect the number of body grooming episodes (Table 2; Fig. 5). In contrast, Ast primarily increased the number of episodes ($P < 0.01$) and the time spent in body grooming ($P = 0.03$; Table 2; Fig. 5).

Discussion

We investigated in the present study the effect of Ucn1 administration into SON on the behaviour in the OFT. This procedure was initially described in a study investigating the emotionality of rats (Hall 1934) and is thought to provide an elegant approach to measure a variety of parameters including anxiety-like behaviours, displacement behaviour and sedation (Prut and Belzung 2003). Ucn1 is known to induce a time-dependent anxiety-like behaviour after intracerebroventricular administration in a battery of tests that are thought to allow the measurement of conditioned and unconditioned anxiety-related behaviour in rats (Spina et al. 2002). Our data show that Ucn1 administration into the SON at doses lower than or equal to 0.01 µg produce anxiety-like behaviour, whereas higher doses failed to induce such effect. This inverted U-shaped dose–response curve is typical for neuropeptide action (Bohus et al. 1978; Greenberg et al. 2002). On the basis of our findings, the maximal effective dose of 0.01 µg Ucn1 was selected for the second experiment.

Pre-treatment with Ast blocked the Ucn1-induced increase in the latency for the first visit of the open field centre without causing significant effects on general locomotion (Fig. 3). This reveals that Ast antagonises Ucn1 effects in a way that can be interpreted as anxiolysis (Prut and Belzung 2003). This suggests that activation of CRF_2R by synthetic Ucn1 in the SON area modifies risk assessment behaviour.

We also observed a significant effect of Ast pre-treatment on Ucn1-induced grooming (time, episodes and latency; Fig. 4). Auto-grooming is a maintenance behaviour that serves predominantly functions such as cleaning and temperature regulation in many small mammals (Jolles et al. 1979). However, an intensified grooming behaviour can be also observed in response to stressor exposure, to the administration of selected psychotropic drugs and in some mouse mutants (Kalueff and Tuohimaa 2005). Several regions appear to be involved in the regulation of grooming including the hypothalamus (Kalueff and Tuohimaa 2005). It has been proposed that Ucn1 might act as paracrine and autocrine regulator in the hypothalamus after dendritic and somatic release (Arima and Aguilera 2000) and a recent report confirms the presence of Ucn1 immunoreactivity in dendrites (Swinny et al. 2002). In addition, evidence supports that dendritic release of peptides can generate hormone-like signal (Ludwig and Leng 2006), implying a possibility that peptide reaches more

Table 2 Effect of Intra-SON Ucn1 administration in the presence of the CRF_2R antagonist Ast, on distinct elements of the grooming response (weighted mean ± SEM)

	Saline	Ucn1	Ast	Ast + Ucn1
(a) Time (s)				
Head washing	37.09 ± 4.871	54.92 ± 8.193*	48.14 ± 6.204	25.99 ± 3.557#
Body grooming	15.50 ± 2.488	50.84 ± 2.209*	49.47 ± 9.205*	9.27 ± 4.541
(b) Number of episodes				
Head washing	10.20 ± 1.443	14.58 ± 2.226*	10.32 ± 1.308	5.50 ± 1.066#
Body grooming	4.11 ± 0.354	7.89 ± 0.354	12.30 ± 2.227*	4.18 ± 0.775

* $P < 0.05$ compared to saline, # $P < 0.05$ compared to 0.01 µg Ucn1 ($n = 8$ per treatment)

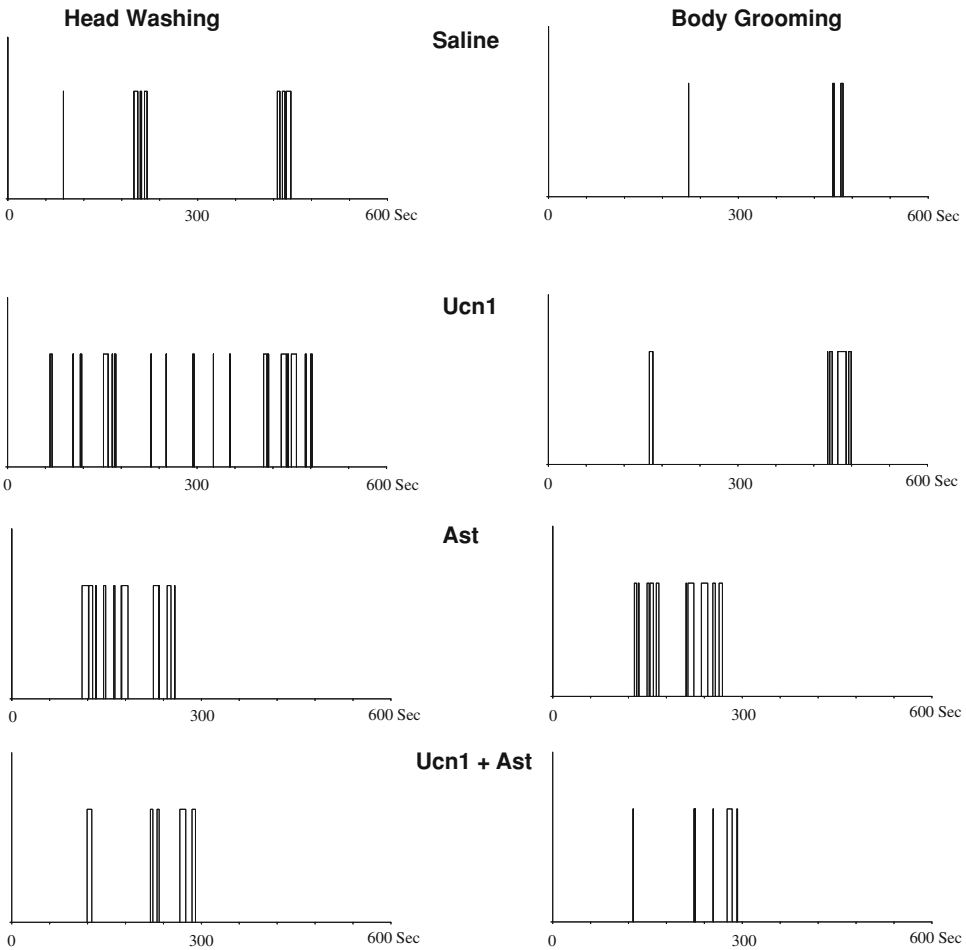

Fig. 5 Event plot of grooming behaviour of a representative animal in the OFT after intra-SON treatments (Ucn1 = 0.01 µg Urocortin1; Ast = 0.1 µg Astressin-2B). The *X* axis represents time duration (600 s) for which animal was placed in the open field. Both Ucn1 and Ast differentially affected the grooming behaviour in animals. Ucn1 increased the head washing and the number of prematurely terminated grooming bouts. In contrary, Ast increased both head washing and body grooming similarly

remote brain area sites known to be involved in grooming behaviour, including the lateral hypothalamus in biologically relevant concentrations (Lammers et al. 1987).

It is reasonable to infer from the data obtained in our study that treatment of Ast per se increases grooming behaviour (Fig. 4). However, a closer examination of the distinct grooming elements reveals that in the Ucn1-, but not Ast-treated group the number of head washing episodes are two times higher than that of body grooming, providing an evidence for the presence of prematurely terminated grooming bouts (Fig. 5). These data demonstrate that Ucn1 treatment affected grooming differentially than Ast injections. It has been reported that distinct grooming elements are sensitive to the level of stress indicated by the fact that pronounced stress and an anxious state of the animal predominantly trigger rostral grooming (including head washing; (Kalueff and Tuohimaa 2004; van Erp et al. 1995). This suggests that the emotional constituents underlying the increased grooming differ between Ucn1 and Ast. It seems plausible that Ucn1 administration into SON elicits a high arousal state in

rats when they are tested in the open field whereas the effects induced by Ast alone require further investigation. However, from our findings it is difficult to discriminate that these effects are due to changed appetitive motivational state or altered stress response or both. It is important to note that when Ast was administered as pre-treatment to Ucn1, there was a reduction in overall grooming without dissociation between the number of head washing and body grooming episodes. Since Ast is a competitive selective blocker of CRF_2R, it is likely that the higher available concentration of Ucn1 after exogenous administration displaces the Ast from receptor and normalizes a response to that observed under basal conditions. These data are in agreement with other observations showing that activation of CRF_2R can regulate the behavioural output in dynamic manner, having opposing effects depending on the concentration of agonist available at the receptor site. In fact it has been described as higher concentrations, available either by direct infusion or through manipulation of induced stress, activate neuronal activity while lower concentrations inhibit it (Henry et al. 2006; Pernar et al. 2004). Likewise in our study, it is possible that while a disinhibition of CRF_2R by Ast induced an anxiety-like behaviour (via removal of low Ucn1 concentration-mediated inhibition of CRF_2R), higher concentrations up to 0.01 μg activated CRF_2R resulting in high arousal state. However, an increase in Ucn1 concentration above 0.01 μg might have induced a receptor trafficking leading to a loss of responsiveness (Reyes et al. 2006).

Taken together, our results show that administration of Ucn1 at doses lower than 0.01 μg into SON affects OFT behaviour in rats. This behaviour is reversed by the pre-treatment of a selective CRF_2R antagonist. The current findings suggest that the Ucn1 originating from the SON may play a role in controlling the animal's state of arousal.

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