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Anxiogenic and antinociceptive effects induced by corticotropin-releasing factor (CRF) injections into the periaqueductal gray are modulated by CRF1 receptor in mice

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ABSTRACT

Chemical or electrical stimulation of the dorsal portion of the midbrain periaqueductal gray (dPAG) produces anxiogenic and antinociceptive effects. In rats, chemical stimulation of dPAG by local infusion of the neuropeptide corticotropin-releasing factor (CRF) provokes anxiogenic effects in the elevated plus-maze test (EPM). CRF also produces antinociception when injected intracerebroventricularly in rats, however it remains unclear whether this response is also observed following CRF injection into the dPAG in mice. Yet, given that there are CRF1 and CRF2 receptor subtypes within the PAG, it is important to show in which receptor subtypes CRF exert its anxiogenic and antinociceptive effects in the dPAG. Here, we investigated the role of these receptors in the anxiogenic (assessed in the EPM) and antinociceptive (assessed by the Formalin test: 2.5% formalin injection into the right hind paw) effects following intra-dPAG infusion of CRF in mice. The results show that intra-dPAG injections of CRF (75 pmol/0.1 μl and 150 pmol/0.2 μl) produced dose-dependent anxiogenic and antinociceptive effects. In addition, local infusion of NBI 27914 (5-chloro-4-(N-(cyclopropyl) methyl-N-propylamino)-2-methyl-6-(2,4,6-trichlorophenyl)-aminopyridine; 2 nmol/0.2 μl), a CRF1 receptor antagonist, completely blocked both the anxiogenic and antinociceptive effects induced by local infusion of CRF, while that of antisauvagine 30 (ASV30; 1 nmol/0.2 μl), a CRF2 receptor antagonist, did not alter the CRF effects. Present results are suggestive that CRF1 (but not CRF2) receptors play a crucial role in the anxiogenic and antinociceptive effects induced by CRF in the dPAG in mice.

1. Introduction

The dorsal periaqueductal gray matter (dPAG) is a midbrain site markedly involved in fear/anxiety-evoked responses as well as in nociception (see, for example, Bandler and Carrive, 1988; Deakin and Graeff, 1991; Fardin et al., 1984a,b; Litvin et al., 2007). Chemical or electrical stimulation of dPAG elicits defensive behavior such as freezing, flight and fight reaction, escalation of risk assessment behavior and arousal (e.g., Bandler and Carrive, 1988; Schenberg et al., 2005) and autonomic activation (e.g., tachycardia, hypertension, tachypnea — Bandler et al., 1991; Hayward et al., 2003; McDougall et al., 1985). These responses are generally accompanied by antinociception (e.g., Coimbra and Brandão, 1997; Fanselow, 1991).

Among the various neurotransmitter systems pointed out to play a role in the mediation of defensive and antinociceptive responses elicited by environmentally aversive stimuli, the neuropeptide corticotropin-releasing factor or hormone (CRF or CRH) has attracted the interest of many researchers investigating its role in the modulation of defensive reactions (Baldwin et al., 1991; Berridge and Dunn, 1989; Carvalho-Netto et al., 2007; Litvin et al., 2007; Stenzel-Poore et al., 1994). CRF is a 41-amino acid peptide that activates the hypothalamo–pituitary–adrenal (HPA) axis, releasing, at the end of a cascade, glucocorticoids from the adrenal gland. HPA axis hyperactivation has been related to several brain disturbances such as anxiety disorders, depression, epilepsy and drug addiction (Allen et al., 2011; Kanner, 2011; Lim et al., 2011; Pariante and Lightman, 2008). Besides its action on the HPA axis, CRF also acts in other brain areas such as the amygdala (Carrasco and Van de Kar, 2003; Shekhar et al., 2005), bed nucleus of stria terminalis (Sahuque et al., 2006), locus coeruleus (Chen et al., 1992), dorsal raphe nucleus (Carrasco and Van de Kar, 2003) and PAG (Borelli and Brandão, 2008; Martins et al., 1997) increasing anxiety-like responses in various animal tests.

Regarding the involvement of CRF receptors located within the PAG on fear/anxiety-related responses, previous studies have shown that intra-dPAG infusion of h/rCRF (Martins et al., 2000) and ovine CRF (Borelli and Brandão, 2008) increase anxiety-like behaviors in

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rats exposed to the elevated plus maze (EPM), a widely used animal test of anxiety (Handley and Mithani, 1984; Lister, 1987; Pellow et al., 1985). In mice, Carvalho-Netto et al. (2007) have shown that intra-PAG infusion of ovine CRF increases avoidance behavior in two different anxiety tests, the mouse defense test battery (MDTB) and the rat exposure test (RET).

It has been emphasized that CRF can activate Gs-protein-coupled CRF1 or CRF2 receptors triggering the cAMP-PKA cascade pathway (Chang et al., 1993; Chen et al., 1993; Lovenberg et al., 1995; Perrin et al., 1995; Vita et al., 1993). Moreover, both receptor subtypes have been found strongly expressed in different PAG columns, where a high density of CRFergic neurons have also been reported (Merchenthaler, 1984; Steckler and Holsboer, 1999; Swanson et al., 1983). In addition, Bowers et al. (2003) have related CRF microinjection into the PAG with increased neuron firing and excitatory activity. However, it remains unclear at which receptor subtypes (CRF1, CRF2 or both) CRF produces anxiety-like responses in rodents. Evidence showing that CRF1 receptors play an important role in the modulation of defensive responses has been reported in several studies. For instance, intra-PAG (Litvin et al., 2007) or intracerebroventricular (Tezval et al., 2004) infusions of CRF1 receptor agonist, cortagine, produce anxiogenic-like effects in various animal tests of anxiety. On the other hand, intracerebral injection of CRF2 receptor antagonist (e.g., antisauvagine-30) has been related to both anxiogenic and anxiolytic effects (Kishimoto et al., 2000; Takahashi et al., 2001). However, as far as we know there are no studies comparing the anxiogenic effects of CRF at CRF1 and CRF2 receptors within the PAG.

Different types of environmentally induced antinociception have been reported in a wide range of species (e.g., Behbehani, 1995; Bolles and Fanselow, 1980; Harris, 1996; Millan, 2002; Rodgers, 1995). According to Bolles and Fanselow (1980), fear and pain are independent and competing motivational systems implicated in distinct biological functions. In this context, besides inducing defensive reactions, systemic or intracerebroventricular injections of CRF also elicit antinociception (e.g., Bogdanov and Yarushkina, 2007; Lariviere and Melzack, 2000). However, it remains unknown whether CRF1 or CRF2 receptors located within the midbrain PAG play a role in the antinociceptive effect of CRF.

In the present study, we investigated the role played by CRF1 and CRF2 receptors located within the mouse PAG on the anxiogenic and antinociceptive effects produced by local infusion of CRF. To block CRF receptors, we used the selective CRF1 and CRF2 receptor antagonists, respectively, NBI 27914 ((5-chloro-4-(N-cyclopropyl)methyl-N-propyl)-2-methyl-6-(2,4,6-trichlorophenyl) aminopyridine) (Hammack et al., 2003; Jochman et al., 2005), and antisauvagine-30 (Sahuque et al., 2006; Takahashi et al., 2001).

2. Materials and methods

2.1. Subjects

Subjects were 101 male adult Swiss mice (Univ. Estadual Paulista — UNESP, SP, Brazil) weighing 25–35 g at testing. Mice were housed in groups of 10 per cage (size: 41×34×16 cm) and maintained under a normal 12-h light cycle (lights on at 7:00 am) in a temperature- and humidity-controlled environment (23±2 °C/R.H. 55±5%). Food and water were freely available except during the brief test periods. All mice were naïve at the beginning of the experiments.

2.2. Drugs

Drugs used were: corticotropin-releasing factor (CRF — 75 and 150 pmol; Sigma-Aldrich, Brazil); NBI 27914 [5-chloro-4-(N-cyclopropyl)methyl-N-propyl)-2-methyl-6-(2,4,6-trichlorophenyl) aminopyridine], a selective CRF1 receptor antagonist (2 nmol; Tocris Cookson Inc., Ballwin, USA); antisauvagine 30, a selective CRF2 antagonist (1 nmol; Tocris Cookson Inc., Ballwin, USA). The doses used were based on previous studies (Hammack et al., 2003; Jochman et al., 2005; Martins et al., 1997; Risbrough et al., 2003; Sahuque et al., 2006, Takahashi et al., 2001). NBI was dissolved in dimethylsulfoxide (80% DMSO in physiological saline). CRF and antisauvagine-30 were dissolved in physiological saline (0.9% NaCl).

2.3. Surgery and microinjection

Stainless steel guide cannulae (7 mm long, 26-gauge; Insight Equipamentos Científicos Ltd., Brazil) were implanted in mice anesthetized by intraperitoneal injection of ketamine (80 mg/kg) plus xylasin (8 mg/kg). The guide cannula was fixed to the skull with dental acrylic and jeweler's screws. Stereotaxic coordinates (Paxinos and Fraklin, 2001) for the PAG were, respectively, 4.1 mm posterior to bregma, 1.3 mm lateral to the midline, and 2.2 mm ventral to the skull surface, the guide cannula being angled at 26° to the vertical. A dummy cannula (33-gauge stainless steel wire; Fishtex Industry and Commerce of Plastics Ltd.), inserted into the guide cannula immediately after surgery, served to reduce the incidence of occlusion.

Five to seven days after surgery, the various solutions were injected into the PAG through microinjection units (33-gauge stainless steel cannula; Insight Equipamentos Científicos Ltd., Brazil), which extended 1.0 mm beyond the tip of the guide cannula. Each microinjection unit was attached to a 5-μl Hamilton microsyringe via polyethylene tubing (PE-10), and drug administration was controlled by an infusion pump (BI 2000, Insight Equipamentos Científicos Ltd., Brazil) programmed to deliver 0.2 μl over a period of 60 s. The microinjection procedure consisted of gently restraining the animal, removing the dummy cannula, inserting the injection unit which remained in situ for a further 60 s, following the injection. Confirmation of successful infusion was obtained by monitoring the movement of a small air bubble in the PE-10 tubing.

2.4. Elevated plus maze and behavioral analysis

The basic EPM design was very similar to that originally described by Lister (1987) and comprised two open arms (30×5×0.25 cm) and two closed arms (30×5×15 cm) connected via a common central platform (5×5 cm). The apparatus was constructed from wood (floor) and transparent glass (clear walls) and was raised to a height of 38.5 cm above floor level.

After each drug treatment (see Section 2.6 General procedure) in the PAG (Fig. 1), each mouse was placed in an individual holding cage and then transported to the maze. Testing commenced by placing the subject on the central platform of the maze (facing an open arm), after which the experimenter immediately withdrew to an adjacent laboratory. The videotaped test sessions were 5 min in duration and, between subjects, the maze was thoroughly cleaned with 20% alcohol. All experiments were performed under normal laboratory illumination (1×60 W yellow incandescent lamp positioned approximately 1.80 m above the EPM floor), during the light phase of the light–dark cycle.

Videotapes were scored by a highly trained observer using an ethological analysis package developed by Dr. Morato's group at Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, University of São Paulo (personal communication). Behavioral parameters comprised both conventional spatiotemporal (Experiments 1–3) and ethological (Experiments 2 and 3) measures (Rodgers and Johnson, 1995). Conventional measures were the frequencies of closed arm entries (arm entry = all four paws into an arm), percent open entries [(open/total)×100)], and percent spent in open arms of the maze [e.g., (time open/300)×100]. Ethological measures comprised frequency scores for open arm end exploration (OAEE: entering the open arm 10-cm distal section from the central square), head dipping (HD: exploratory movement of head/shoulders over the side

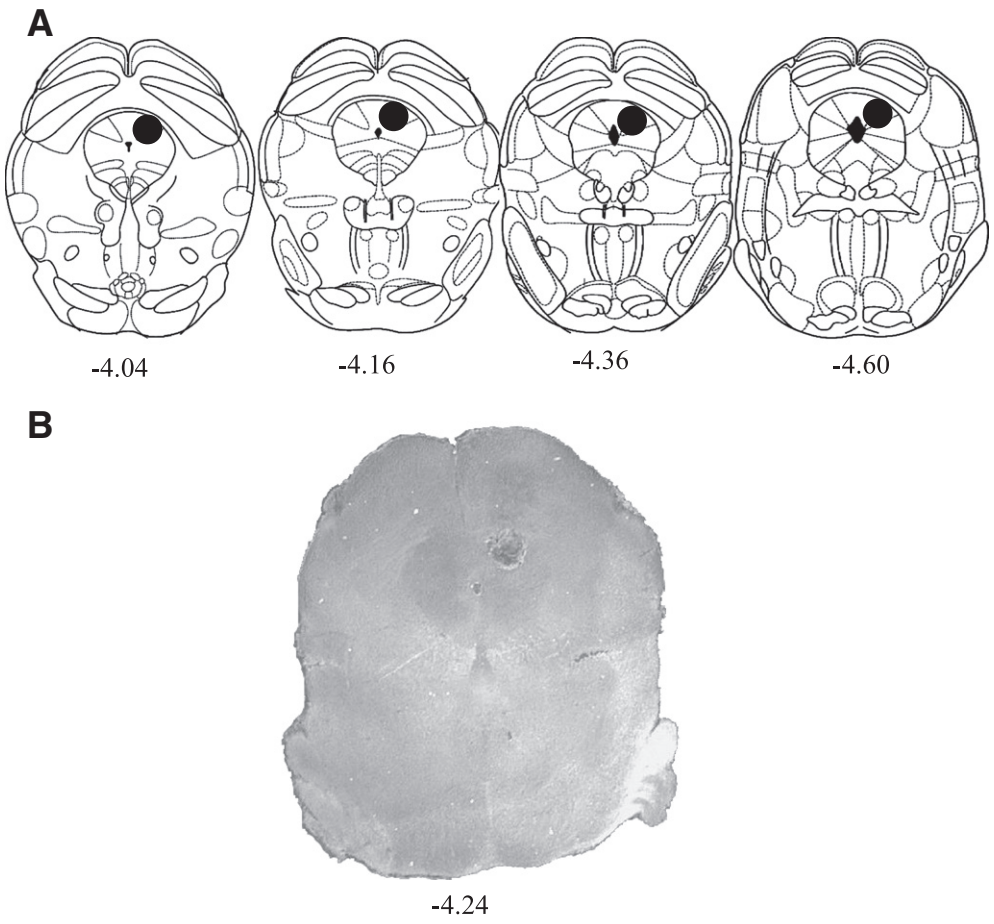

Fig. 1. (A) A schematic representation of microinfusion sites within the midbrain dorsal periaqueductal gray (dPAG) of the mouse. Black circle corresponds to whole area in which the various microinjections were placed in each slice (distance from bregma in mm), taken from the Paxinos and Fraklin (2001) Atlas. (B) Photomicrograph of midbrain coronal section from a representative subject, showing an injection site into the dPAG. Section corresponds to −4.24 mm from bregma in the atlas of Paxinos and Fraklin (2001).

of the maze) and stretched-attend postures (SAP: exploratory posture in which the body stretched forward then retracted to the original position without any forward locomotion). In view of importance of thigmotactic cues to patterns of plus-maze exploration (Treit et al., 1993) head dipping and SAP were further differentiated as a function of whereabouts on the maze they were displayed. Consistent with earlier reports (Rodgers and Johnson, 1995) the closed arms and the central platform were together designated "protected" areas (i.e., offering relative security), while the open arms were designated "unprotected" areas. Data for the HD and SAP measures are reported both as protected and unprotected scores.

2.5. Nociception analysis

Nociception was assessed by the formalin test as previously described (Abbott et al., 1995). The formalin test causes a two-phase nociceptive response (Dubuisson and Dennis, 1977). The first phase begins immediately after formalin injection and lasts approximately 5 min. It results from the direct stimulation of nociceptors (Dubuisson and Dennis, 1977; McCall et al., 1996). The second phase begins 20 min after the injection and lasts approximately 40 min (Bon et al., 2002). In the present study, 50 µl of formalin (2.5%) was injected into the dorsal surface of the right hind paw of the mouse, which was placed in a glass holding cage (30 cm × 20 cm × 25 cm). Nociceptive response was recorded by measuring the time (in seconds) spent on licking the paw injected with formalin.

2.6. General procedure

2.6.1. Experiment 1: Effects of intra-PAG injection of CRF on anxiety-like indices and nociception

2.6.1.1. Anxiety test. Five to six days after surgery, mice were transported to the experimental room and left undisturbed for at least 30 min prior to testing (this procedure was followed in all three experiments). Each mouse then received a microinjection (0, 75 or 150 pmol) of CRF in the PAG and 10 min later it was placed on the EPM and conventional spatiotemporal measures (see Section 2.4 above) were recorded for 5 min.

2.6.1.2. Nociception test. Formalin was injected into the hind paw of mice 48 h after the EPM test and 25 min later they received an intra-PAG injection of CRF (0, 75 or 150 pmol) and placed in the glass holding cage for 10 min to record the time spent on licking the injected paw (25–35 min after formalin injection).

2.6.2. Experiment 2: Effects of combined intra-PAG injections with NBI 27914, a CRF1 receptor antagonist, and CRF on anxiety-like indices and nociception

2.6.2.1. Anxiety test. NBI 27914 (0 or 2 nmol) was microinjected into the PAG of the mice (pretreatment) and 10 min later they received a (0 or 150 pmol) microinjection of CRF into the PAG (treatment). After

a further 10 min, each mouse was placed on the EPM to record both conventional spatiotemporal and ethological measures (see Section 2.4 above) for a period of 5 min.

2.6.2.2. Nociception test. Formalin was injected into the hind paw of the mice, 48 h after the EPM test, and 5 min later they received an intra-PAG injection of NBI 27914 (pretreatment; 0 or 2 nmol) and, 10 min later, an injection of CRF (treatment; 0 or 150 pmol). Ten minutes later, mice were individually placed in the glass holding cage for 10 min, to record the time spent on licking the injected paw (25–35 min after formalin injection).

2.6.3. Experiment 3: Effects of combined intra-PAG injections with antisauvagine 30 (ASV 30), a CRF2 receptor antagonist, and CRF on anxiety-like indices and nociception

2.6.3.1. Anxiety test. Mice received a microinjection of ASV 30 (0 or 1 nmol) into the PAG (pretreatment) and 10 min later they received a CRF (0 or 150 pmol) microinjection into the PAG (treatment). After 10 min, each mouse was placed on the EPM, to record both conventional spatiotemporal and ethological measures (see Section 2.4 above) for a period of five minutes.

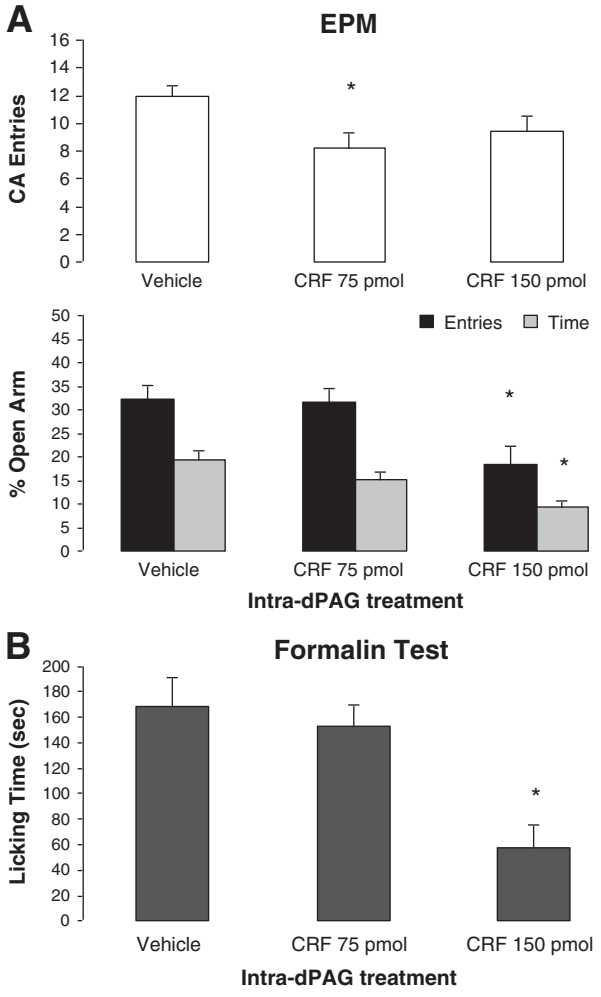

Fig. 2. (A) Effects of CRF microinjection (0, 75 or 150 pmol) into the dPAG upon frequency of closed arm (CA) entries (upper panel) as well as on percent of open-arm entries and percent of open-arm time (lower panel) in the EPM. Bars represent means (± SEM). N = 9–15. *p<0.05 compared to vehicle group. (B) Effects of CRF microinjection (0, 75 or 150 pmol) into the dPAG on time (in seconds) spent on licking the formalin injected paw in mice. Bars represent means (± SEM). N = 8–13. *p<0.05 compared to vehicle group.

2.6.3.2. Nociception test. Formalin was injected into the hind paw of the mice, 48 h after the EPM test, and 5 min later they received an intra-PAG injection of ASV 30 (pretreatment; 0 or 1 nmol) and, 10 min later, an injection of CRF (treatment; 0 or 150 pmol). Ten minutes later, mice were individually placed in the glass holding cage for 10 min, to record the time spent on licking the injected paw (25–35 min after formalin injection).

Importantly, all animals of Experiments 1–3 that had received intra-dPAG injection of CRF on day 1 (EPM test) received saline on day 3 (Nociception test) and vice versa.

2.7. Histological analysis

At the end of testing, all animals received a 0.1 µl intra-PAG infusion of 1% Evans blue by the same microinjection procedure as for the drugs. Animals were then sacrificed by anesthetic overdose, their brains removed and injection sites checked histologically by reference to the atlas of Paxinos and Fraklin (2001). Data from animals with injection sites outside the PAG were excluded from the study.

2.8. Statistical analysis

All results were initially submitted to Levene's test for homogeneity of variance. Where Levene's test yielded significant heterogeneity, results were transformed to the log, square root or cube root and then confirmed for homogeneity of variance before being subjected to one- or two-way analysis of variance (ANOVA). Finally, data were subjected to the post hoc Duncan test. In Experiment 1, one-way ANOVA was carried out. In Experiments 2 and 3, two-way ANOVA was carried out (factor 1, pretreatment; factor 2, treatment). In all cases, a P value ≤ 0.05 was required for significance.

2.9. Ethics

The experimental protocols were conducted according to the ethical principles of the Brazilian College of Animal Experimentation (COBEA) and approved by the local Research Ethics Committee (CEP/FCF/Car-UNESP: protocol number 10/2006).

3. Results

3.1. Brain injection site

Fig. 1 shows a schematic representation of microinfusion sites within the midbrain dorsal periaqueductal gray (dPAG) and a photomicrograph of a midbrain coronal section of a representative subject, showing an injection site into the dPAG of the mouse.

3.2. Experiment 1: Effects of intra-dPAG injection of CRF on anxiety-like indices and nociception

Fig. 2A shows the frequency of closed arm entries and anxiety-like indices recorded for a 5-min period in the EPM in mice microinjected with CRF (0, 75 or 150 pmol) into the dPAG. One-way ANOVA followed by Duncan test revealed that CRF (150 pmol) decreased the percentage of open-arm entries ($F_{2,34} = 5.38$; $p<0.05$) and percentage of open-arm time ($F_{2,34} = 7.03$; $p<0.05$). In addition, one-way ANOVA revealed significant differences in closed-arm entries ($F_{2,34} = 3.29$; $p<0.05$) and the post-hoc test showed that CRF (75 pmol) decreased the closed-arm entries.

Fig. 2B shows the time spent on licking the paw injected with 2.5% formalin in mice treated with CRF (0, 75 or 150 pmol) into the dPAG. One-way ANOVA revealed significant differences between groups ($F_{2,28} = 9.2$; $p<0.05$) and post-hoc comparisons (Duncan's test) revealed that CRF (150 pmol) decreased the time spent on licking the paw injected with formalin.

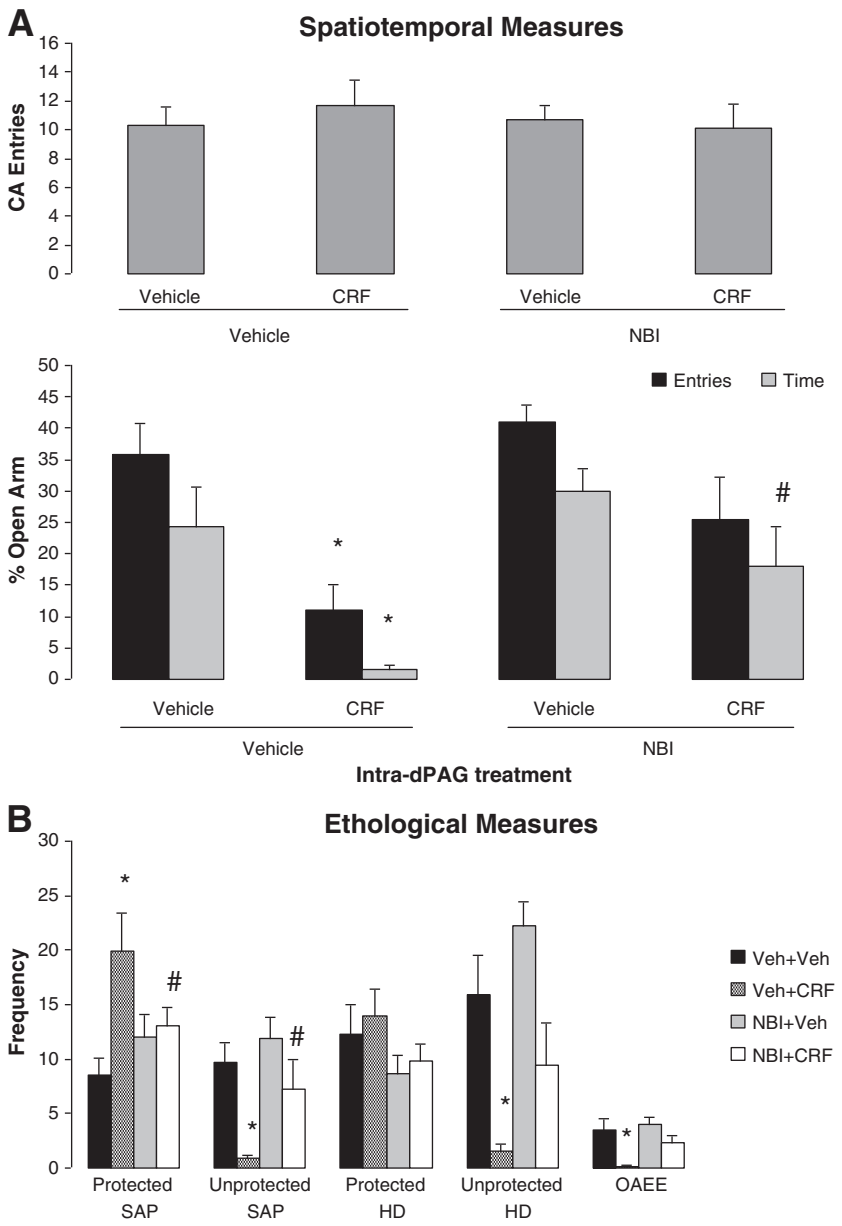

Fig. 3. Effects of combined intra-dPAG microinjections of NBI 27914 (0 or 2 nmol) and CRF (0 or 150 pmol) on the (A) spatiotemporal measures [frequency of closed-arm (CA) entries (upper panel), percent of open-arm entries and percent of open-arm time (lower panel)] and (B) ethological measures [frequency of protected and unprotected stretched-attend postures (SAP), protected and unprotected head dipping (HD), and open arm end exploration (OAEE)] in mice exposed to the EPM. Bars represent means (± SEM). N = 9–14. $^{*,\#}p<0.05$ compared to control group (veh + veh) and to veh + CRF group, respectively.

3.3. Experiment 2: Effects of combined intra-dPAG injections of NBI 27914 and CRF on anxiety-like indices and nociception

Fig. 3A shows the effects of combined injections of NBI 27914 (0 or 2 nmol) and CRF (0 or 150 pmol) into the PAG on the behavior of mice exposed in the EPM. Two-way ANOVA revealed significant differences for the NBI 27914 and CRF treatments on% open-arm entries (NBI: $F_{1,37}=4.22$; $p<0.05$; CRF: $F_{1,37}=15.16$; $p<0.05$) and% open-arm time (NBI: $F_{1,37}=4.06$; $p<0.05$; CRF: $F_{1,37}=9.84$; $p<0.05$), but did not reveal a significant effect for the NBI × CRF interaction on either index (% open arm entries: $F_{1,37}=1.06$; $p=0.31$;% open arm time: $F_{1,37}=0.98$; $p=0.33$). Post-hoc comparisons confirmed the results of Exp. 1, demonstrating that CRF decreased the% open-arm entries and% open-arm time ($p<0.05$), compared to control (veh + veh). Duncan's test also revealed that the NBI 27914 injection selectively blocked the CRF effects on both anxiety indices. Also, ANOVA did not reveal any effect for the NBI ($F_{1,37}=0.13$; $p=0.72$) and CRF ($F_{1,37}=0.16$; $p=0.69$) treatments or NBI versus CRF interactions ($F_{1,37}=0.39$; $p=0.54$) on closed-arm entries (Fig. 3A).

Consistent with the above observations, analysis also revealed some significant changes in the ethological measures, namely protected SAP (CRF: $F_{1,37}=6.86$, $p<0.05$; NBI x CRF interaction: $F_{1,37}=4.82$, $p<0.05$), unprotected SAP (NBI: $F_{1,37}=4.47$, $p<0.05$; CRF: $F_{1,37}=11.73$, $p<0.01$), unprotected HD (NBI: $F_{1,37}=5.01$, $p<0.05$; CRF: $F_{1,37}=18.24$, $p<0.01$); OAEE (CRF: $F_{1,37}=8.79$, $p<0.01$) (Fig. 3B). Post-hoc comparisons indicated that, relative to the veh + veh control group, veh + CRF increased protected SAP ($p<0.01$) and decreased unprotected SAP ($p<0.01$), unprotected HD ($p<0.01$) and OAEE ($p<0.05$). Interestingly, post-hoc Duncan test revealed that the NBI + CRF group decreased the effects produced by CRF (veh + CRF) on protected and unprotected SAP ($p<0.05$). Also, NBI + CRF group tended to reduce CRF effects on unprotected HD

($p = 0.09$) and OAEE ($p = 0.09$). Finally, protected HD was not significantly affected by any of the treatments ($F_{1,37} = 2.72$, $p = 0.11$) (Fig. 3B).

Fig. 4 shows the effects of combined injections of NBI 27914 (0 or 2 nmol) and CRF (0 or 150 pmol) into the dPAG on the time spent on licking 2.5% formalin-injected paw. Two-way ANOVA did not reveal any differences for the NBI 27914 treatment ($F_{1,32} = 2.49$; $p = 0.12$), but showed significant differences for the CRF treatment ($F_{1,32} = 10.45$; $p < 0.05$) and a tendency toward an effect for the NBI versus CRF interaction ($F_{1,32} = 3.51$; $p = 0.07$). Post-hoc comparisons revealed that CRF (veh + CRF) reduced the time spent on licking the injected paw, compared to the control group (veh + veh). However, intra-dPAG NBI 27914 blocked the antinociceptive effect of CRF (NBI + CRF vs veh + CRF; $p < 0.05$) without provoking any effect alone (NBI + veh vs veh + veh; $p = 0.83$).

3.4. Experiment 3: Effects of combined intra-dPAG injections of antisauvagine 30 (ASV 30), a CRF2 receptor antagonist, and CRF on anxiety-like indices and nociception

Fig. 5A shows the effects of combined injections of ASV 30 (0 or 1 nmol) and CRF (0 or 150 pmol) into the dPAG on the behavior of mice exposed in the EPM. Two-way ANOVA did not reveal any significant difference for the ASV 30 treatment on% open-arm entries ($F_{1,42} = 0.02$; $p = 0.89$) or% open-arm time ($F_{1,42} = 0.09$; $p = 0.77$), but showed a significant effect for the CRF treatment on both indices (% open-arm entries: $F_{1,42} = 26.48$; $p < 0.05$;% open-arm time: $F_{1,42} = 28.65$; $p < 0.05$). No effect of ASV 30 × CRF interaction were revealed on either anxiety index (% open-arm entries: $F_{1,42} = 0.05$, $p = 0.82$;% open-arm time: $F_{1,42} = 0.08$; $p = 0.78$). Post-hoc comparisons confirmed the CRF effects in decreasing% open-arm entries and% open-arm time ($p < 0.05$), compared to control (veh + veh), and revealed that ASV 30 did not change the CRF effects in either anxiety-like index. Also, ANOVA did not reveal any effect for the ASV 30 pretreatment ($F_{1,42} = 0.26$; $p = 0.61$), CRF treatment ($F_{1,42} = 2.47$; $p = 0.12$) or ASV 30 versus CRF interaction ($F_{1,42} = 0.35$; $p = 0.56$) on closed-arm entries (Fig. 5A).

As shown in Fig. 5B, ANOVA also revealed some significant effects for CRF treatment in the ethological measures (protected SAP: $F_{1,42} = 20.59$, $p < 0.01$; unprotected SAP: $F_{1,42} = 16.88$, $p < 0.01$; unprotected HD: $F_{1,42} = 20.56$, $p < 0.01$; OAEE: $F_{1,42} = 14.18$, $p < 0.01$). Post-hoc comparisons confirmed that both veh + CRF and ASV 30 + CRF groups showed higher frequency in protected SAP ($p < 0.01$) and lower frequency in unprotected SAP ($p < 0.05$), unprotected HD ($p < 0.01$) and OAEE ($p \leq 0.05$) compared to veh + veh control group.

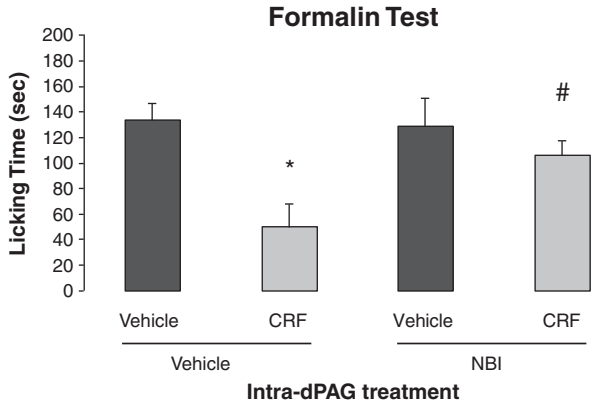

Fig. 4. Effects of combined intra-dPAG microinjections of NBI 27914 (0 or 2 nmol) and CRF (0 or 150 pmol) on the time (in seconds) spent on licking the formalin-injected paw. Bars represent means (± SEM). N = 8–11. $^{*,\#}p < 0.05$ compared to control group (veh + veh) and to veh + CRF group, respectively.

Protected HD was not significantly affected by any of the treatments ($F_{1,42} \leq 0.50$, $p = 0.48$) (Fig. 5B).

Fig. 6 shows the effects of combined injections of ASV 30 (0 or 1 nmol) and CRF (0 or 150 pmol) into the PAG on time spent on licking the 2.5% formalin-injected paw. Two-way ANOVA did not reveal significant differences for the ASV 30 treatment ($F_{1,33} = 1.49$; $p = 0.23$) or for the ASV 30 × CRF interaction ($F_{1,33} = 0.05$; $p = 0.83$), but showed significant differences for the CRF treatment ($F_{1,33} = 33.34$; $p < 0.05$). Post-hoc comparisons revealed that CRF (veh + CRF) reduced the time spent licking the formalin-injected paw, compared to the control group (veh + veh), an effect that was not blocked by intra-dPAG pretreatment with ASV 30.

4. Discussion

The main results of the present study showed that intra-dPAG injections of CRF enhanced anxiety-like behavior in the EPM and inhibited the nociceptive response induced by formalin injection into the paw in mice. In addition, prior intra-dPAG injections of CRF1 (but not CRF2) receptor antagonist attenuated both the anxiogenic and antinociceptive effects produced by CRF injection into this limbic midbrain structure.

Intra-dPAG microinjections of CRF reduced open arm exploration without affecting general activity (closed-arm entries) in the EPM. This result corroborates previous studies demonstrating that intra-PAG CRF enhances anxiety-like indices in the EPM in rats (Borelli and Brandão, 2008; Martins et al., 1997) as well as in mouse anxiety tests such as the mouse defense test battery (MDTB) and rat exposure test (RET, Carvalho-Netto et al., 2007). Here, the anxiogenic effects of CRF (i.e., reduction in% open-arm entries and% open-arm time) were completely blocked by a prior intra-dPAG injection of NBI 27914, a CRF1 receptor antagonist. Importantly, closed-arm entries, a widely used measure of general activity level (e.g., Cruz et al., 1994; Rodgers and Johnson, 1995), remained unchanged in animals treated with NBI 27914, combined either with CRF or with vehicle, suggesting that the NBI 27914 effects were selective in reducing the anxiogenic effects produced by CRF. The reduction in the closed-arm entries observed with the lower dose of CRF (Fig. 2A) seems to be an isolated effect, since the higher dose of the neuropeptide did not confirm this effect, either when injected alone (Fig. 2A) or combined with vehicle (Figs. 3A and 5A). Indeed, in addition to preventing the anxiogenic-like effects of intra-dPAG CRF on direct measures of open arm exploration (see above), intra-dPAG NBI 27914 also prevented the anxiogenic-like increase in protected SAP and reduction in unprotected SAP as well as tended to reduce unprotected HD and open arm end exploration seen in response to CRF treatment. Importantly, intra-dPAG NBI 27914 was completely devoid of intrinsic behavioral activity under present test conditions. Taken together, the present results suggest that the antianxiety effect following deletion of CRF1 receptor gene in mice observed elsewhere (Contarino et al., 1999; Smith et al., 1998; Timpl et al., 1998) might be at least in part due to a dysfunction of PAG CRF1 receptor activity.

Intra-dPAG injections of CRF also reduced the nociceptive response induced by formalin injection into the hind paw. CRF-treated animals showed a reduction in the time spent on licking the formalin-injected paw, characterizing an antinociceptive role played by this neuropeptide in the mouse PAG. Presently, we do not know how CRF elicits antinociception in the PAG. Possibly, CRF activates neurons that belong to the descending inhibitory system of pain (Fields and Basbaum, 1999), which, in turn, inhibit nociceptive afferents in the dorsal horn of the spinal cord (Harris, 1996; Kharkevich and Churukanov, 1999; Millan, 2002). Similar to what was observed in anxiety-like indices, intra-dPAG injection of NBI 27914 completely blocked the antinociceptive effect of CRF, suggesting, for the first time, that CRF1 receptors located within this midbrain structure play a role in the modulation of nociception.

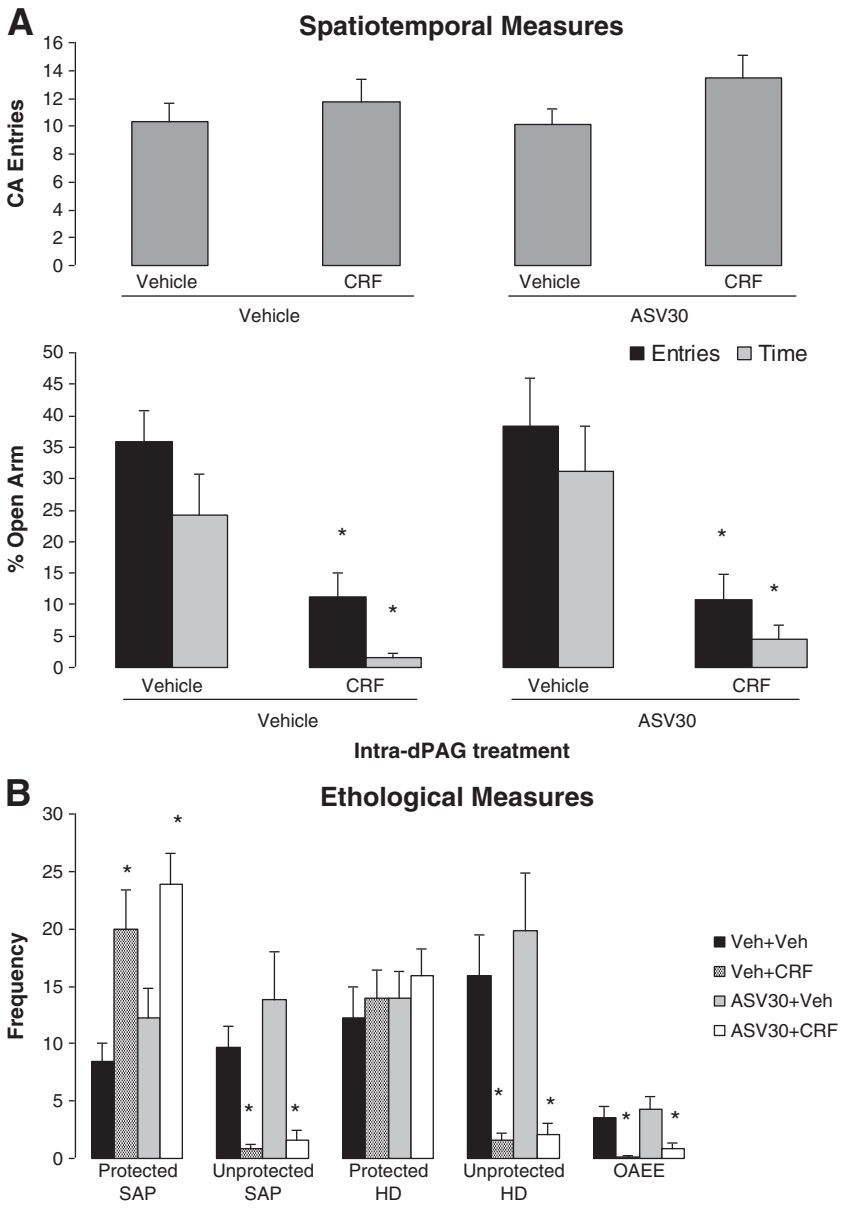

Fig. 5. Effects of combined intra-dPAG microinjections of ASV 30 (0 or 1 nmol) and CRF (0 or 150 pmol) on the (A) spatiotemporal measures [frequency of closed-arm (CA) entries (upper panel), percent of open-arm entries and percent of open-arm time (lower panel)] and (B) ethological measures [frequency of protected and unprotected stretched-attend postures (SAP), protected and unprotected head dipping (HD), and open arm end exploration (OAEE)] in mice exposed to the EPM. Bars represent means (± SEM). N = 9–14. *$p<0.05$ compared to control group (veh + veh).

Both anxiogenic and antinociceptive effects produced by CRF seem to be selective for the CRF1 receptor, since intra-dPAG microinjection of ASV 30, a CRF2 receptor antagonist, altered neither the anxiety nor the antinociception response. Intra-dPAG ASV 30 was also unable to prevent the anxiogenic (observed in both conventional and ethological anxiety-related measures) and antinociceptive effects produced by local infusion of CRF. Thus, despite their presence in all columns of the PAG (Steckler and Holsboer, 1999), CRF2 receptors do not play a role in anxiety, assessed in the EPM, or in nociception assessed in the formalin test in mice.

Regarding the effects of CRF on anxiety, Sahuque et al. (2006) have demonstrated that this neuropeptide also enhances anxiety when injected into the bed nucleus of the stria terminalis (BNST), a brain structure that has also been related to defensive behavior (Casada and Dafny, 1991; Chen et al., 2009; Walker et al., 2003). Those authors also demonstrated that intra-BNST injections of a CRF1 (but not CRF2) receptor antagonist prevented the anxiogenic effect produced by CRF. Importantly, Sahuque et al. (2006) demonstrated that intra-BNST ASV 30, at the same dose employed in the present study, was able to prevent place aversion induced by local CRF injection, but did not attenuate the anxiogenic effect produced by this neuropeptide in rats exposed to the EPM. In view of these findings, it seems that the emotional state elicited by EPM exposure does not recruit CRF2 receptors located within the BNST (Sahuque et al., 2006) and dPAG (present results) in rats and mice, respectively.

It has been demonstrated that CRF has a higher affinity for CRF1 receptors than for CRF2 receptors (Vaughan et al., 1995). This differential affinity might be the main reason for the presently observed lack of effect of ASV 30 on the anxiogenic and antinociceptive effects produced by CRF. However, it has been reported that the anxiogenic-like effects produced with i.c.v. injections of CRF (200 pmol) in mice exposed to the startle paradigm were attenuated with i.c.v. injection of ASV 30 (Risbrough et al., 2003), at a similar dose to that used in the present study. Therefore, it is likely that the dose of

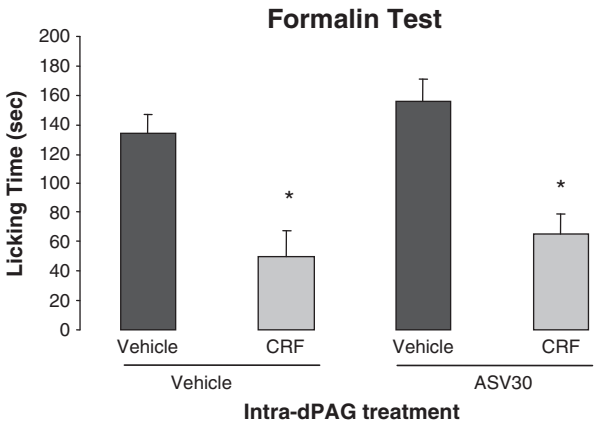

Fig. 6. Effects of combined intra-dPAG microinjections of ASV 30 (0 or 1 nmol) and CRF (0 or 150 pmol) on time (in seconds) spent on licking the formalin-injected paw. Bars represent means (± SEM). N = 8–11. *p<0.05 compared to control group (veh + veh).

150 pmol of CRF, which was directly injected into the dPAG, has been sufficiently high to activate CRF2 receptors in the present study. Moreover, while i.c.v. injection of a selective CRF1 receptor agonist, stressin-1A, produces anxiogenic-like effects in rats exposed to the shock probe test, no effects were observed with urocortine III, a selective CRF2 receptor agonist (Zhao et al., 2007). These inconsistent results indicate a need for further studies with different animal models of anxiety to better understand the role of CRF2 receptors in the neurobiology of emotional states. In this context, the present results indicate that the PAG CRF1 (but not CRF2) receptor is an important target on which CRF exerts its anxiogenic-like actions in mice exposed to the EPM.

Finally, it has been emphasized that the PAG receives CRF-containing neuron projections from other anxiety-related brain structures such as amygdala, hypothalamus and BNST (Gray and Magnuson, 1992). Furthermore, studies *in vitro* have shown an excitatory effect on PAG neurons following CRF administration (Bowers et al., 2003). Thus, the CRF anxiogenic and antinociceptive actions observed in the present study might be associated with a neuron-firing response as a consequence of membrane depolarization provoked by this neuropeptide within the PAG. Although both CRF1 and CRF2 receptors have been found in the PAG (Merchenthaler, 1984; Swanson et al., 1983), present results suggest that the anxiogenic and the antinociceptive effects of CRF introduced into the dPAG are related to CRF1 but not CRF2 receptor activation. However, given the contrasting effects observed with manipulation of CRF2 receptors in emotional tests (Bale et al., 2000; Kishimoto et al., 2000; Radulovic et al., 1999; Risbrough et al., 2003; Takahashi et al., 2001; Zhao et al., 2007), it is important to point out that further studies are needed to determine the role played by this CRF receptor subtype in the nociception response as well as in anxiety-related behavior.

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References

Abbott, F.V., Franklin, K.B., Westbrook, R.F., 1995. The formalin test: scoring properties of the first and the second phases of the pain response in rats. Pain 60 (1), 91–102.
Allen, C.D., Lee, S., Koob, G.F., Rivier, C., 2011. Immediate and prolonged effects of alcohol exposure on the activity of the hypothalamic-pituitary-adrenal axis in adults and adolescent rats. Brain Behav. Immun. 25 (1), S50–S60.
Baldwin, H.A., Rassnick, S., Rivier, J., Koob, G.F., Britton, K.T., 1991. CRF antagonist reverses the anxiogenic response to ethanol withdrawal in the rat. Psychopharmacology 103 (2), 227–232.
Bale, T.L., Contarino, A., Smith, G.W., Chan, R., Gold, L.H., Sawchenko, P.E., Koob, G.F., Vale, W.W., Lee, K.F., 2000. Mice deficient for corticotropin-releasing hormone receptor-2 display anxiety-like behaviour and are hypersensitive to stress. Nat. Genet. 24 (4), 410–414.
Bandler, R., Carrive, P., 1988. Integrated defense reaction elicited by excitatory amino acid in the midbrain periaquedutal gray region of the unrestrained cat. Brain Res. 439 (1–2), 95–106.
Bandler, R., Carrive, P., Zhang, S.P., 1991. Integration of somatic and autonomic reactions within the midbrain periaqueductal grey: viscerotopic, somatotopic and functional organization. Prog. Brain Res. 87, 267–305.
Behbehani, M.M., 1995. Functional characteristics of the midbrain periaqueductal gray. Prog. Neurobiol. 46, 575–605.
Berridge, C.W., Dunn, A.J., 1989. CRF and restraint stress decrease exploratory behavior hypophisectomized mice. Pharmacol. Biochem. Behav. 34 (3), 517–519.
Bogdanov, A.I., Yarushkina, N.I., 2007. The role of hormones of the hypothalamo–hypophyseal–adrenocortical system in the analgesic effect of corticotropin-releasing hormone. Neurosci. Behav. Physiol. 37 (4), 363–367.
Bolles, R.C., Fanselow, M.S., 1980. A perceptual–defensive–recuperative model of fear and pain. Behav. Brain Sci. 3, 291–322.
Bon, K., Wilson, S.G., Mogil, J.S., Roberts, W.J., 2002. Genetic evidence for the correlation of deep dorsal horn Fos protein immunoreactivity with tonic formalin pain behavior. J. Pain 3 (3), 181–189.
Borelli, K.G., Brandão, M.L., 2008. Effects of ovine CRF injections into the dorsomedial, dorsolateral and lateral columns of the periaqueductal gray: a functional role for the dorsomedial column. Horm. Behav. 53 (1), 40–50.
Bowers, L.K., Swisher, C.B., Behbehani, M.M., 2003. Membrane and synaptic effects of corticotropin-releasing factor on periaqueductal gray neurons of the rat. Brain Res. 981, 52–57.
Carrasco, G.A., Van de Kar, L.D., 2003. Neuroendocrine pharmacology of stress. Eur. J. Pharmacol. 463, 235–272.
Carvalho-Netto, E.F., Litvin, Y., Nunes-de-Souza, R.L., Blanchard, D.C., Blanchard, R.J., 2007. Effects of intra-PAG infusions of ovine CRF on defensive behaviors in Swiss-Webster mice. Behav. Brain Res. 176 (2), 222–229.
Casada, J.H., Dafny, N., 1991. Restraint and stimulation of bed nucleus of the stria terminalis produce similar stress-like behavior. Brain Res. Bull. 27 (2), 207–212.
Chang, C.P., Pearse 2nd, R.V., O'Connel, 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.
Chen, M.F., Chiu, T.H., Lee, E.H., 1992. Noradrenergic mediation of the memory-enhancing effect of corticotropin-releasing factor in the locus coeruleus of rats. Psychoneuroendrocrinology 17 (2–3), 113–124.
Chen, R., Lewis, K.A., Perrin, M.W., Vale, W.W., 1993. Expression cloning of a human corticotropin-releasing factor receptor. Proc. Natl. Acad. Sci. U S A 90, 8967–8971.
Chen, W., Shields, J., Huang, H., King, J.A., 2009. Female fear: influence of estrus cycle on behavioral response and neuronal activation. Behav. Brain Res. 201 (1), 8–13.
Coimbra, N.C., Brandão, M.L., 1997. Effects of 5-HT2 receptors blockade on fear-induced analgesia elicited by electrical stimulation of the deep layers of the superior colliculus and dorsal periaqueductal gray. Behav. Brain Res. 87 (1), 97–103.
Contarino, A., Dellu, F., Koob, G.F., Smith, G.W., Lee, K.F., Vale, W., Gold, L.H., 1999. Reduced anxiety-like and cognitive performance in mice lacking the corticotropin-releasing factor receptor 1. Brain Res. 835 (1), 1–9.
Cruz, A.P., Frei, F., Graeff, F.G., 1994. Ethopharmacological analysis of rat behavior on the elevated plus maze. Pharmacol. Biochem. Behav. 49 (1), 171–176.
Deakin, J.F.W., Graeff, F.G., 1991. 5-HT and mechanisms of defense. Psychopharmacology 5, 305–315.
Dubuisson, D., Dennis, S.G., 1977. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine and brain stimulation in rats and cats. Pain 4, 161–174.
Fanselow, M.S., 1991. The midbrain periaqueductal gray as a coordinator of action in response to fear and anxiety. In: Depaulis, A., Bandler, R. (Eds.), The Midbrain Periaqueductal Gray Matter. Plenum Press, New York, pp. 151–173.
Fardin, V., Oliveras, J.L., Besson, J.M., 1984a. A reinvestigation of the analgesic effects induced by stimulation of the periaqueductal gray matter in the rat I. The production of behavioral side effects together with analgesia. Brain Res. 306 (1–2), 105–123.
Fardin, V., Oliveras, J.L., Besson, J.M., 1984b. A reinvestigation of the analgesic effects induced by stimulation of the periaqueductal gray matter in the rat II. Diferential characteristics of the analgesia induced by ventral and dorsal PAG stimulation. Brain Res. 306 (1–2), 125–139.
Fields, H.L., Basbaum, A.I., 1999. Central nervous system mechanisms of pain modulation. In: Wall, P.D., Melzack, R. (Eds.), Textbook of Pain. Churchill Livingstone, New York, pp. 206–217.
Gray, T.S., Magnuson, D.J., 1992. Peptide immunoreactive neurons in the amygdala and the bed nucleus of the stria terminalis project to the midbrain central gray in the rat. Peptides 13 (3), 451–460.
Hammack, S.E., Schmid, M.J., LoPresti, M.L., Der-Avakian, A., Pellymounter, L.A., Forster, A.C., Watkins, L.R., Maier, S.F., 2003. Corticotropin-releasing hormone type 2 receptor in the dorsal raphé nucleus mediate the behavioral consequence of incontrolable stress. J. Neurosci. 23 (3), 1019–1025.
Handley, S.L., Mithani, S., 1984. Effects of alpha-adrenoceptor agonist and antagonist in a maze-exploration model of fear-motivated behaviour. Naunyn Schmiedebergs Arch. Pharmacol. 327 (1), 1–5.
Harris, J.A., 1996. Descending antinociceptive mechanisms in the brainstem: their role in the animal's defensive system. J. Physiol. 90, 15–25.

Hayward, L.F., Schwartz, C.L., Davenport, P.W., 2003. Respiratory response to activation or disinhibition of the dorsal periaqueductal gray in rats. J. Appl. Physiol. 94 (3), 913–922.
Jochman, K.A., Newman, S.M., Kalin, N.H., Bakshi, V.P., 2005. Corticotropin-releasing factor-1 receptors in the basolateral amygdala mediate stress-induced anorexia. Behav. Neurosci. 119 (6), 1448–1458.
Kanner, A.M., 2011. Depression and epilepsy: a bidirectional relation? Epilepsia 52, 21–27.
Kharkevich, D.A., Churukanov, V.V., 1999. Pharmacological regulation of descending cortical control of the nociceptive processing. Eur. J. Pharmacol. 375 (1–3), 121–131.
Kishimoto, T., Radulovic, J., Radulovic, M., Lin, C.R., Schrick, C., Hooshmand, F., Hermanson, O., Rosenfeld, M.G., Spiess, J., 2000. Deletion of crhr2 reveals an anxiolytic role for corticotropin-releasing hormone receptor-2. Nat. Genet. 24, 415–419.
Lariviere, W.R., Melzack, R., 2000. The role of corticotropin-releasing factor in pain and analgesia. Pain 84 (1), 1–12.
Lim, L.W., Blockland, A., van Duinem, M., Visser-Vanderwalle, V., Tan, S., Vlamings, L., Janssen, M., Jahanshahi, A., Aziz-Mohammadi, M., Steinbusch, H.W.M., Schruers, K., Temel, Y., 2011. Increased plasma corticotestorone levels after periaqueductal gray stimulation-induced escape reaction or panic attacks in rats. Behav. Brain Res. 218, 301–307.
Lister, R.G., 1987. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology 92, 180–185.
Litvin, Y., Pentkowiski, N.S., Blanchard, D.C., Blanchard, R.J., 2007. CRF type 1 receptors in the dorsal periaqueductal gray modulate anxiety-induced defensive behaviors. Horm. Behav. 52 (2), 244–251.
Lovenberg, T.W., Liaw, C.W., Grigoriadis, D.E., Clevenger, W., Chalmers, D.T., De Souza, E.B., Oltersdorf, T., 1995. Cloning and characterization of a functionally distinct corticotropin-releasing factor receptor subtype from rat brain. Proc. Natl. Acad. Sci. U S A 92, 836–840.
Martins, A.P., Marras, R.A., Guimarães, F.S., 1997. Anxiogenic effect of corticotropin-releasing hormone in the dorsal periaqueductal gray. Neuroreport 8, 3601–3604.
Martins, A.P., Marras, R.A., Guimarães, F.S., 2000. Anxiolytic effect of a CRH receptor antagonist in the dorsal periaqueductal gray. Depress. Anxiety 12, 99–101.
McCall, W.D., Tanner, K.D., Levine, J.D., 1996. Formalin induces biphasic activity in C-fibers in the rat. Neurosci. Lett. 208 (1), 41–48.
McDougall, A., Dampney, R., Bandler, R., 1985. Cardiovascular components of the defence reaction evoked by excitation of neuronal cell bodies in the midbrain periaqueductal grey of the cat. Neurosci. Lett. 60 (1), 69–75.
Merchenthaler, I., 1984. Corticotropin releasing factor (CRF)-like immunoreactivity in the rat central nervous system. Extrahypothalamic distribution. Peptides 5, 53–69.
Millan, M.J., 2002. Descending control of pain. Prog. Neurobiol. 66, 355–474.
Pariante, C.M., Lightman, S.L., 2008. The HPA axis in major depression: classical theories and new developments. Trends Neurosci. 31 (9), 464–468.
Paxinos, G., Franklin, K.B.J. (Eds.), 2001. The mouse brain in stereotaxic coordinates. Academic Press, California, USA.
Pellow, S., Chopin, P., File, S.E., Briley, M., 1985. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Methods 14, 149–167.
Perrin, M., Donaldson, C., Chen, R., Blount, A., Berggren, T., Bilezikjian, L., Sawchenko, P., Vale, W., 1995. Identification of a second corticotropin-releasing factor receptor gene and characterization of a cDNA expressed in heart. Proc. Natl. Acad. Sci. U S A 92, 2969–2973.
Radulovic, J., Ruhmann, A., Liepold, T., Spiess, J., 1999. Modulation of learning and anxiety by corticotropin-releasing factor (CRF) and stress: differential roles of CRF receptors 1 and 2. J. Neurosci. 19 (12), 5016–5025.
Risbrough, V.B., Haugher, L.R., Pelleymounter, M.A., Geyer, M.A., 2003. Role of corticotropin-releasing factor (CRF) receptors 1 and 2 in CRF-potentiated acoustic startle in mice. Psychopharmacology 170, 178–187.
Rodgers, R.J., 1995. Neuropharmacological aspects of adaptative pain inhibition in murine victims of aggression. Aggress. Behav. 21, 29–39.
Rodgers, J.R., Johnson, N.J., 1995. Factor analysis of spatiotemporal and ethological measures in the murine elevated plus-maze test of anxiety. Pharmacol. Biochem. Behav. 52 (2), 297–303.
Sahuque, L.L., Kullberg, E.F., Mcgeehan, A.J., Kinder, J.R., Hicks, M.P., Blanton, M.G., Janak, P.H., Olive, M.F., 2006. Anxiogenic and aversive effects of corticotropin-releasing factor (CRF) in the bed nucleus of the stria terminalis in the rat: role of CRF receptor subtypes. Psychopharmacology (Berl) 186 (1), 122–132.
Schenberg, L.C., Póvoa, R.M., Costa, A.L., Caldellas, A.V., Tufik, S., Bittencourt, A.S.L., 2005. Functional specializations within the tectum defense systems of the rat. Neurosci. Biobehav. Rev. 29, 1279–1298.
Shekhar, A., Truitt, W., Rainnie, D., Sajdyk, T., 2005. Role of stress, corticotrophin releasing factor (CRF) and amygdala plasticity in chronic anxiety. Stress 8 (4), 209–219.
Smith, G.W., Aubry, J.M., Dellu, F., Contarino, A., Bilezikjian, L.M., Gold, L.H., Chen, R., Marchuk, Y., Hauser, C., Bentley, C.A., Sawchenko, P.E., Koob, G.F., Vale, W., Lee, K.F., 1998. Corticotropin releasing factor receptor 1-deficient mice display decreased anxiety, impaired stress response, and aberrant neuroendocrine development. Neuron 20, 1093–1102.
Steckler, T., Holsboer, F., 1999. Corticotropin-releasing hormone receptor subtypes and emotion. Biol. Psychiatry 46, 1480–1508.
Stenzel-Poore, M.P., Heinrichs, S.C., Rivest, S., Koob, G.F., Vale, W.W., 1994. Over-production of corticotopin-releasing factor in transgenic mice: a genetic model of anxiogenic behavior. J. Neurosci. 14, 2579–2584.
Swanson, L.W., Sawchenko, P.E., Rivier, J., Vale, W.W., 1983. Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study. Neuroendocrinology 36, 165–186.
Takahashi, L.K., Ho, S.P., Livanov, V., Graciani, N., Arneric, S.P., 2001. Antagonism of CRF(2) receptors produces anxiolytic behavior in animal models of anxiety. Brain Res. 902, 135–142.
Tezval, H., Jahn, O., Todorovic, C., Sasse, A., Eckart, K., Spiess, J., 2004. Cortagine, a specific agonist of corticotropin-releasing factor receptor subtype 1, is anxiogenic and antidepressive in the mouse models. Proc. Natl. Acad. Sci. U S A 101 (25), 9468–9473.
Timpl, P., Spanagel, R., Sillaber, I., Kresse, A., Reul, J.M., Stalla, G.K., Blanquet, V., Steckler, T., Holsboer, F., Wurst, W., 1998. Impaired stress response and reduced anxiety in mice lacking a functional corticotropin-releasing hormone receptor 1. Nat. Genet. 19, 162–166.
Treit, D., Menard, J., Royan, C., 1993. Anxiogenic stimuli in the elevated plus-maze. Pharmacol. Biochem. Behav. 44, 463–469.
Vaughan, J., Donaldson, C., Bittencourt, J., Perrin, M.H., Lewis, K., Sutton, S., Chan, R., Turnbull, A.V., Lovejoy, D., Rivier, C., Rivier, J., Sawchenko, P.E., Vale, W., 1995. Urocortin, a mammalian neuropeptide related to fish urotensin I and to corticotropin releasing factor. Nature 378, 287–292.
Vita, N., Laurent, P., Lefort, S., Chalon, P., Lelias, J.M., Kaghad, M., Le Fur, G., Caput, D., Ferrara, P., 1993. Primary structure and functional expression of mouse pituitary and human brain corticotropin-releasing factor receptor. FEBS Lett. 335, 1–5.
Walker, D.L., Toufexis, D.J., Davis, M., 2003. Role of the bed nucleus of the stria terminalis versus the amygdala in fear, stress and anxiety. Eur. J. Pharmacol. 463, 199–216.
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 (3), 846–854.