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Interactions between corticotropin-releasing factor and the serotonin 1A receptor system on acoustic startle amplitude and prepulse inhibition of the startle response in two rat strains

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ABSTRACT

Both the neuropeptide, corticotropin-releasing factor (CRF) and the serotonin 1A (5-HT_{1A}) receptor systems have been implicated in anxiety disorders and there is evidence that the two systems interact with each other to affect behavior. Both systems have individually been shown to affect prepulse inhibition (PPI) of the acoustic startle response. PPI is a form of sensorimotor gating that is reduced in patients with anxiety disorders including post-traumatic stress and panic disorder. Here, we examined whether the two systems interact or counteract each other to affect acoustic startle amplitude, PPI and habituation of the startle response. In experiment 1, Brown Norway (BN) and Wistar-Kyoto (WKY) rats were administered ether an intraperitoneal (IP) injection of saline or the 5-HT_{1A} receptor agonist, 8-OH-DPAT 10 min prior to receiving an intracerebroventricular (ICV) infusion of either saline or CRF (0.3 μg). In a second experiment, rats were administered either an IP injection of saline or the 5-HT_{1A} receptor antagonist, WAY 100,635 10 min prior to receiving an ICV infusion of saline or CRF. Thirty min after the ICV infusion, the startle response and PPI were assessed. As we have previously shown, the dose of CRF used in these experiments reduced PPI in BN rats and had no effect on PPI in WKY rats. Administration of 8-OH-DPAT alone had no effect on PPI in either rat strain when the data from the two strains were examined separately. Administration of 8-OH-DPAT added to the effect of CRF in BN rats, and the combination of 8-OH-DPAT and CRF significantly reduced PPI in WKY rats. CRF alone had no effect on baseline startle amplitude in either rat strain, but CRF enhanced the 8-OH-DPAT-induced increase in startle in both strains. Administration of WAY 100,635 did not affect the CRF-induced change in PPI and there were no interactions between CRF and WAY 100,635 on baseline startle. The results suggest that activation of the 5-HT_{1A} receptor can potentiate the effect of CRF on endophenotypes of anxiety disorders in animal models.

This article is part of a Special Issue entitled 'Anxiety and Depression'.

1. Introduction

1.1. Corticotropin-releasing factor and serotonin in anxiety models

Corticotropin-releasing factor (CRF), a 41-amino acid peptide, is synthesized in hypothalamic (Vale et al., 1981) and extra-hypothalamic brain regions including the central nucleus of the amygdala, hippocampus, and frontal cortex (Swanson et al., 1983). The neuropeptide acts as both a hypothalamic releasing hormone and a neurotransmitter to affect endocrine, autonomic, and behavioral responses to stress (Bale and Vale, 2004; Gray, 1993). There are two CRF G protein-coupled receptors, CRF_1 and CRF_2 (Chang et al., 1993; Lovenberg et al., 1995), which are expressed in brain regions known to modulate startle amplitude and prepulse inhibition (PPI) of the startle response, including the basolateral amygdala, hippocampus, and frontal cortex (Swerdlow et al., 2001; Van Pett et al., 2000). Additionally, there is a projection from CRF-containing neurons in the central nucleus of the amygdala to the major dopaminergic, serotonergic and noradrenergic nuclei (see Gray, 1993 for review). Levels of CRF in cerebrospinal fluid are higher in patients with post-traumatic stress disorder (PTSD) than in controls (Baker et al., 1999; Bremner et al., 1997; Sauter et al., 2003).

Within 10 years of the identification of CRF, the anxiogenic effect of the peptide was convincingly demonstrated (Britton et al., 1986; Dunn and Berridge, 1990; Dunn and File, 1987). Over the past 30

Abbreviations: CRF, Corticotropin-Releasing Factor.

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years, a considerable body of evidence has demonstrated that CRF has behavioral effects that are independent of HPA axis activation. Both intracerebroventricular (ICV) (Jones et al., 1998; Spina et al., 2002) and intra-bed nucleus of the stria terminalis (BNST) infusion of CRF (Sahuque et al., 2006) increase anxiety-like behavior. Further, CRF over-expressing mice show more anxiety-like behavior (Van Gaalen et al., 2002; Stenzel-Poore et al., 1994), and CRF_1 receptor knockout (KO) mice show less anxiety-like behavior than wild type (WT) mice (Contarino et al., 1999; Smith et al., 1998; Timpl et al., 1998). Conditional KO of the CRF_1 receptor in the limbic system only is anxiolytic even though the pituitary CRF receptor system is intact (Muller et al., 2003). In some studies, the CRF_2 receptor also appears to mediate an anxiogenic effect of CRF (Takahashi, 2001). However, CRF_2 receptor KO mice show more anxiety-like behavior than WT mice (Bale et al., 2000). It appears that the two receptors mediate opposing effects of CRF on some behaviors (Kishimoto et al., 2000; Risbrough et al., 2004).

The serotonin 5-HT_{1A} receptor system has also been shown to have a role in animal models of anxiety and to be altered in clinical anxiety. 5-HT_{1A} receptors are located on both soma and dendrites of serotonergic neurons in the raphe nuclei, as well as on post-synaptic membranes in cortico-limbic brain regions (see Barnes and Sharp, 1999 for review). The results of a number of studies reveal that mice lacking 5-HT_{1A} receptors show more anxiety-like behavior that WT controls (Heisler et al., 1998; Parks et al., 1998; Ramboz et al., 1998). Additionally, Breese et al., (2004) showed that a 5-HT_{1A} receptor agonist reduces a stress-induced increase in anxiety-like behavior. 5-HT_{1A} receptor binding is reduced in patients with panic disorder (Nash et al., 2008) and social anxiety disorder (Lanzenberger et al., 2007). Together, these data suggest that activation of the 5-HT_{1A} receptor is anxiolytic.

CRF interacts with the serotonergic dorsal raphe nucleus in complex and dynamic ways to affect behavior (see Valentino et al., 2010 for review). Low-dose CRF decreases extracellular concentrations of 5-HT in the lateral striatum (Price et al., 1998) and nucleus accumbens (Lukkes et al., 2008) while relatively high doses increase 5-HT concentrations in these brain regions. However, both low and high doses of CRF decrease the discharge rate of dorsal raphe neurons (Price et al., 1998). Waselus et al., 2011 suggest that CRF has both direct and indirect effects on the dorsal raphe, which might explain why low and high doses of CRF do not have opposing effects on the discharge rate of dorsal raphe neurons. Restraint stress increases extracellular concentrations 5-HT in the central nucleus of the amygdala, and this effect is blocked by a non-selective CRF receptor antagonist (Mo et al., 2008). Further, CRF and a 5-HT_2 receptor agonist act synergistically to increase anxiety-like behavior (Magalhaes et al., 2010). Activation of 5-HT_{1A} receptors can also affect CRF-induced changes in behavior. Selective 5-HT_{1A} receptor agonists attenuate CRF-induced grooming, but do not affect CRF-induced changes in locomotor activity (Lazosky and Britton, 1991). Both CRF and the 5-HT_{1A} receptor system have been individually implicated in anxiety disorders such as PTSD in which the startle response and prepulse inhibition of the response may be altered. However, the combined effects of CRF and drugs that affect the 5-HT_{1A} receptor on these behaviors have not yet been examined.

1.2. The acoustic startle response and prepulse inhibition

The acoustic startle response is a reflexive response to a high-intensity acoustic stimulus with a fast rise-time, and is expressed in whole-body muscle contractions (see Koch and Schnitzler, 1997 for review). Startle amplitude is diminished if the startling stimulus is preceded by a low-intensity, non-startling prepulse stimulus, a phenomenon referred to as prepulse inhibition (PPI) (Hammond et al., 1972). PPI, a measure of sensorimotor gating, is thought to occur so that processing of the prepulse stimulus is not interrupted by the high-intensity startling stimulus (see Koch and Schnitzler, 1997). PPI can be achieved in humans and animals with nearly identical parameters, making it a useful endophenotype for disorders which are characterized by deficits in sensorimotor gating (see Braff et al., 2001). PPI is reduced in patients with anxiety disorders including panic disorder (Ludewig et al., 2002) and post-traumatic stress disorder (PTSD) (Grillon et al., 1998). In PTSD patients, good habituation of the startle response predicts positive treatment outcome (Jaycox et al., 1998; van Minnen and Hagenaars, 2002).

Risbrough and Stein (2006) have discussed evidence suggesting that the startle response and PPI are useful tools for assessing the role of CRF in anxiety disorders. CRF increases acoustic startle amplitude in rats (Liang et al., 1992a; Swerdlow et al., 1989), although this effect is strain-dependent (Conti, 2005; Conti et al., 2002). The effect of CRF on startle amplitude is not altered by lesions of the PVN (Liang et al., 1992b), suggesting that it is not the result of glucocorticoid release. In rats, non-selective CRF antagonists, as well as selective CRF_1 receptor antagonists, attenuate the effect of CRF on startle (Schulz et al., 1996; Swerdlow et al., 1989). In mice, blockade of the CRF_2 receptor, as well as the CRF_1 receptor attenuates the effect of CRF on startle (Risbrough et al., 2003). However, CRF over-expressing mice show a smaller startle response than WT mice (Dirks et al., 2002), perhaps due to other compensatory changes than can occur in transgenic animals. ICV infusion of CRF reduces PPI in both rats (Conti, 2005; Conti et al., 2002; Sutherland et al., 2008; Tejeda et al., 2010) and mice (Risbrough et al., 2004). Bakshi et al., in press find that CRF has long-lasting effects on PPI in rats. Additionally, transgenic mice over-expressing CRF show reduced PPI compared to WT controls (Dirks et al., 2002). Repeated infusion of CRF into the basolateral amygdala, but not the prefrontal cortex, reduces PPI (Bijlsma et al., 2011). In mice, the effect of CRF on PPI in is mediated by the CRF_1 receptor, while the CRF_2 receptor mediates an opposing response (Risbrough et al., 2004). Additionally, the CRF_1 receptor blockade, but not the glucocorticoid receptor blockade attenuates the reduction in PPI seen in CRF over-expressing mice (Groenink et al., 2008). However, we have found that the CRF_1 receptor does not mediate the effect of CRF on PPI in Brown Norway rats (Sutherland and Conti, 2011).

The 5-HT_{1A} receptor agonist, 8-OH-DPAT, has been shown to increase baseline startle amplitude (Davis et al., 1986). However, 5-HT_{1A} receptor knockout mice and wild-type mice show equivalent baseline startle (Dirks et al., 2001). The role of the 5-HT_{1A} receptor in PPI has also been examined. An indolamine hallucinogen which is an agonist at the 5-HT_{1A} receptor decreases PPI and this effect is blocked by the selective 5-HT_{1A} receptor antagonist, WAY 100,635 (Krebs-Thomson et al., 2006). The 5-HT_{1A} receptor agonist, 8-OH-DPAT has also been shown to decrease PPI in rats (Rigdon and Weatherspoon, 1992), although some find that this effect is only seen in rats with high baseline levels of PPI (Gogos and van den Busse, 2007). The effect of 8-OH-DPAT on PPI is attenuated by a selective 5-HT_{1A} receptor antagonist (Sipes and Geyer, 1995). While low-dose 8-OH-DPAT alone decreases PPI in rats, it attenuates the decrease in PPI caused by the NMDA receptor antagonist, MK-801(Bubenikova-Valesove et al., 2007). The role of the 5-HT_{1A} receptor in PPI in mice has also been studied. PPI is equivalent in 5-HT_{1A} receptor knockout and wild-type mice (Dirks et al., 2001). Additionally, maternal separation-induced increases in startle amplitude and decreases in PPI are not attenuated by deletion of the 5-HT_{1A} receptor (Groenink et al., 2011). Never-the-less, 8-OH-DPAT does have effects on PPI in mice. While the 5-HT_{1A} receptor agonist either decreases PPI or has no effect on PPI in rats, the

agonist increases PPI in WT mice, although the effect is strain-dependent (Dulawa et al., 2000; Dulawa and Geyer, 2000). However, 8-OH-DPAT does not affect PPI in 5-HT_{1A} receptor KO mice (Dulawa et al., 2000) suggesting that although the effect of 8-OH-DPAT on mice and rats may not be the same, the 5-HT_{1A} receptor does mediate the effect of 8-OH-DPAT in mice.

The potential interactions between CRF and the 5-HT_{1A} receptor on baseline startle, PPI and startle habituation have not been examined. In the present studies, we assessed such potential interactions by administering the selective 5-HT_{1A} receptor agonist, 8-OH-DPAT or the selective antagonist, WAY 100,635, prior to infusing CRF (ICV). Given that CRF is anxiogenic, that 5-HT_{1A} knockout mice show enhanced anxiety-like behavior and that 5-HT_{1A} agonists are anxiolytic, we sought to assess whether a 5-HT_{1A} agonist would attenuate and a 5-HT_{1A} antagonist would potentiate the effect of CRF on startle amplitude and PPI.

2. Methods

2.1. Animals

Brown Norway (BN) rats (Harlan Sprague-Dawley) and Wistar-Kyoto (WKY) rats (Charles River) were 10 weeks old upon arrival and were maintained on a 12-h light/dark cycle with food and water available *ad libitum*. Rats were group-housed for 1–2 weeks prior to undergoing surgery to have guide cannula aimed at the lateral ventricle for infusion of CRF. Following surgery, the rats were single-housed. All procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Experimental group sizes ranged from 7–10 rats/group. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Stereotaxic surgery and ICV infusion procedure

Rats were anesthetized with isoflurane-in-oxygen (2.0%) and placed in a Kopf stereotaxic instrument equipped with blunt ear bars. The incisor bar was set to −3.0. A stainless steel guide cannula (22 gauge; Plastics One, Roanoke, VA, USA) was aimed at the lateral ventricle (AP -1.0 mm, ML 2.0 mm from Bregma; 4.4 mm ventral from the skull) (Paxinos and Watson, 1986). Two jewelers' screws were placed into the skull and the assembly was held in place with dental cement. A dummy cannula was placed into the guide. Rats were allowed to recover for 5–7 days prior to testing.

For ICV infusion, a 28-gauge cannula attached to PE 20 tubing was inserted into the guide cannula and extended 0.5 mm beyond. A 10.0 μl Hamilton syringe was used to manually deliver saline or CRF (.3 μg in 6.0 μl). The flow of infusate was monitored via introduction of an air bubble into the infusion line. The infusion cannula was kept in place for an additional minute following infusion.

2.3. Startle response and PPI testing

Startle amplitude and PPI were measured in two identical startle chambers (SR-LAB, San Diego Instruments, San Diego, CA, USA) consisting of a nonrestrictive Plexiglas cylinder (9 cm diameter, 18.5 cm length) mounted on a platform located inside a sound- and vibration-attenuating cabinet equipped with a 5-W incandescent bulb and a fan for ventilation. A piezoelectric accelerometer, mounted under each cylinder, detected whole-body startle responses. From the onset of each startle stimulus, output signals from the accelerometer were recorded once/ms for 100 ms by the computer. Signals were rectified, digitized, and stored by the SR-LAB program. Startle response sensitivities were standardized across chambers using a standard calibration tube each day. White noise stimuli were delivered through a horn tweeter controlled by the SR-LAB program.

Rats were placed into a testing chamber for a 5 min acclimation period prior to the delivery of any stimulus. The session was conducted using a 70 dB white noise background. On the first and last 6 trials of the session, a startling stimulus (50 dB above background (or 120 dB0), 40 ms) was presented alone. The remaining trials were presented in a pseudorandom order and included 12 additional trials (middle trials) with the startling stimulus alone (used to calculate % PPI and average startle amplitude), and 12 trials/prepulse stimulus intensity on which a prepulse stimulus (20 ms) preceded the startling stimulus by 100 ms. The prepulse stimuli were 3, 6, 12, 15 or 18 dB above background. Additionally, there were 8 trials on which no stimulus was presented, but activity within the chamber was monitored. The inter-trial interval averaged 20 s. Testing was performed between 10 a.m. and 4 p.m.

2.4. Drug and CRF administration

In Experiment 1, the selective 5-HT_{1A} receptor agonist, 8-OH-DPAT (DPAT; 1.0 mg/kg; IP) or saline was injected 10 min prior to ICV infusion of CRF (.3 μg in 6.0 μl) or saline. This dose of CRF was used because we have previously shown that it reduces PPI in BN, but not in WKY rats (Conti, 2005). Thus, we could examine the effect of DPAT and WAY 100,635 in animals which show an effect of CRF on PPI as well as in those that do not. PPI was tested 30 min after the ICV infusion. In Experiment 2, the selective 5-HT_{1A} receptor antagonist, WAY 100,635 (WAY; 1.0 mg/kg; IP) or saline was injected 10 min before CRF (ICV), and testing began 30 min after the ICV infusion. CRF was a generous gift from Dr. Jean Rivier, The Salk Institute.

2.5. Data analysis

Startle amplitude on the 12 middle trials during which the startling stimulus alone was presented was averaged. These data were used in the analyses of baseline startle amplitude. Percent prepulse inhibition was calculated as 100−100*(average startle amplitude on the prepulse trials/average startle amplitude on the middle startle stimulus alone trials). Percent habituation was calculated as 100−100*(average startle amplitude on the last 6 startle alone trials/average startle amplitude on the first 6 startle alone trials). Percent prepulse inhibition data were analyzed with analysis of variance (ANOVA) with rat strain, IP injection (saline or 5-HT_{1A} compound) and ICV infusion (saline or CRF) as between-subjects factors, and prepulse stimulus intensity as a within-subjects factor. Baseline startle amplitude data and percent habituation data were analyzed using the three between-subjects factors. Data for the 8-OH-DPAT experiment and the WAY 100,635 experiment were subjected to separate analyses.

3. Results

3.1. Experiment 1: Effect of IP injection of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, on CRF-Induced changes in startle and PPI

The effects of CRF and DPAT treatment alone and in combination on PPI are shown in Fig. 1. A four-way ANOVA, with rat strain, IP injection (SAL *vs.* DPAT), ICV infusion (SAL *vs.* CRF) as between-subjects factors, and prepulse stimulus intensity as a within-subjects factor was initially used to test for overall main effects and interactions. The results of this analysis revealed a main effect for rat strain, $F(1, 64) = 12.65$, $p = .001$, with BN rats showing less PPI than WKY rats, a significant main effect for IP injection, $F(1, 64) = 4.92$, $p < .05$, with DPAT-treated rats showing less PPI than SAL-treated rats, and a significant main effect for ICV infusion, $F(1, 64) = 25.5$, $p < .001$, with CRF-treated rats showing less PPI than SAL-treated rats. There were no 2-way interactions between any of these between-subjects factors. There was also a significant effect of prepulse stimulus intensity, $F(4, 256) = 93.3$, $p < .001$. A significant prepulse stimulus intensity X rat strain interaction, $F(4, 256) = 18.0$ $p < .001$, was due to the fact that prepulse intensity had a greater effect on WKY rats than on BN rats. A significant prepulse intensity X IP injection interaction, $F(4, 256) = 3.47$, $p < .01$, was due to the fact that prepulse intensity had a greater effect on SAL-treated than DPAT-treated rats. Finally, a significant prepulse intensity X ICV infusion interaction, $F(4, 256) = 2.85$, $p < .05$, was due to the fact that prepulse intensity had a greater effect on SAL-treated than on CRF-treated rats. There were no 3-way or 4-way interactions involving prepulse intensity.

Inspection of Fig. 1A suggests that both CRF and DPAT alone decreased PPI in BN rats and that the two together had an additive effect resulting in prepulse facilitation. In this strain, there was a significant effect of IP injection, $F(1, 31) = 4.34$, $p < .05$ and a significant effect of ICV infusion, $F(1, 31) = 19.3$, $p < .001$. There was a significant difference between the group treated with SAL (ICV) alone and the group treated with CRF (ICV) alone (SAL/SAL *vs.* SAL/CRF), $F(1, 14) = 11.19$, $p = .005$, while effect of DPAT alone (SAL/SAL *vs.* DPAT/SAL) was not significant, $p > .05$ in BN rats. Nevertheless, the combined treatment of DPAT and CRF reduced PPI over the effect of DPAT alone, $F(1, 17) = 10.5$, $p = .005$, suggesting that the small effect of DPAT added to the effect of CRF. Additionally, the combined treatment with DPAT and CRF reduced PPI compared to

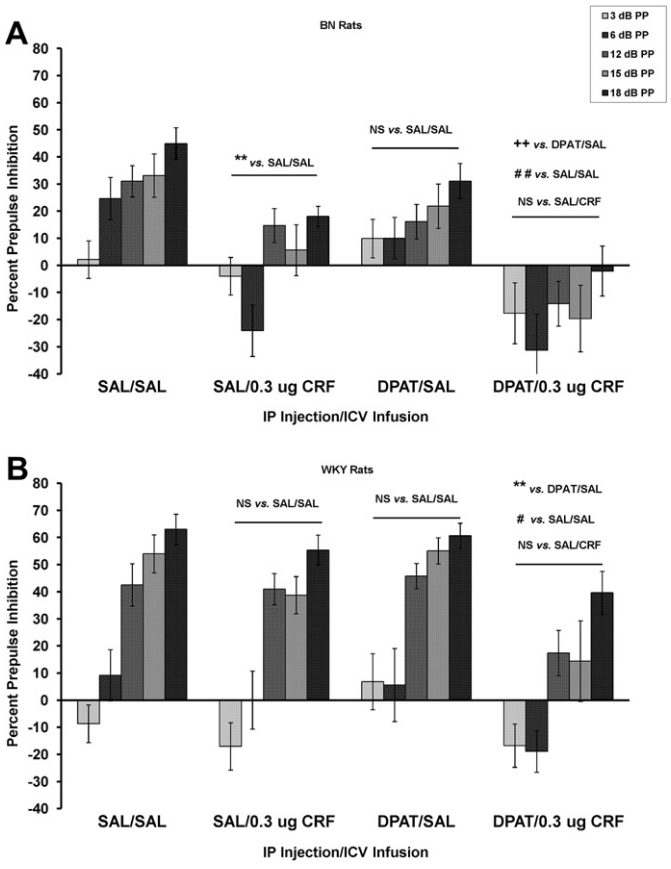

Fig. 1. Percent prepulse inhibition (mean ± SEM) at each of five prepulse stimulus intensities (3–18 dB above background) in BN rats (1A) and WKY rats (1B). Rats received an IP injection of either saline (SAL) or 8-OH-DAT (DPAT) followed 10 min later by an ICV infusion of either SAL or CRF. The results of all analyses are reported in the text. PPI was reduced by CRF alone (SAL/CRF) in the BN rats, $^{**}p = .005$. DPAT alone (DPAT/SAL) had a small, but not significant effect on PPI. The combination of DPAT and CRF (DPAT/CRF) also caused a significant reduction in PPI which was larger than the reduction seen in the SAL/CRF-treated group. In WKY rats (Fig. 1B), neither CRF nor DPAT alone reduced PPI. However, the combination of the two caused a significant reduction in PPI $^{**}p < .01$; $++\ p = .005$; $\#p < .02$; $\#\#p = .001$.

the SAL/SAL control group, $F(1, 16) = 18.4$, $p = .001$, while there was no difference between the combined effect of DPAT/CRF and the effect of CRF alone (SAL/CRF). In WKY rats (Fig. 1B), there was an overall significant effect of CRF on PPI, $F(1, 33) = 7.44$, $p < .01$, but no effect of DPAT. However, inspection of Fig. 1B suggests that neither treatment alone had an effect on PPI in WKY rats, but that together, the two treatments did reduce PPI. Indeed, in WKY rats, there was no effect of CRF on PPI in the absence of DPAT (SAL/SAL *vs.* SAL/CRF), $p > .05$, and no effect of DPAT in the absence of CRF (SAL/SAL *vs.* DPAT/SAL), $p > .05$. However, when the effects of DPAT alone were compared to the combined effects of CRF and DPAT (DPAT/SAL *vs.* DPAT/CRF) a significant difference was seen, $F(1, 16) = 8.76$, $p < .01$. Additionally, PPI was significantly lower in the DPAT/CRF group than in the SAL/SAL group, $F(1, 16) = 6.6$, $p < .02$. However, the combined treatment did not reduce PPI compared to the CRF alone (SAL/CRF) group. Thus, CRF appeared to add to the effect of DPAT.

The effects of CRF and DPAT treatment alone and in combination on baseline startle amplitude are shown in Fig. 2. An overall 3-way ANOVA with rat strain, IP injection and ICV infusion as between-subjects factors revealed a significant effect of IP injection, $F(1, 64) = 25.0$, $p < .001$, with DPAT increasing the startle response. There was also a significant effect of ICV infusion, $F(1, 64) = 6.52$, $p < .02$, with CRF increasing startle amplitude. However, this effect

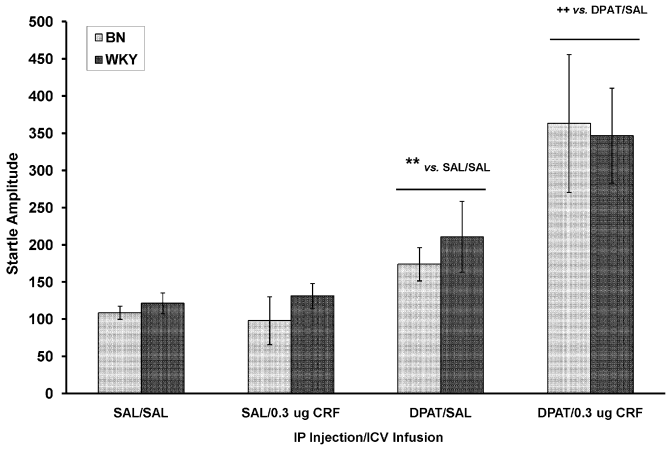

Fig. 2. Baseline startle amplitude (mean ± SEM) in BN and WKY rats on the trials from which the data used to calculate percent PPI were collected. CRF alone (SAL/CRF) did not affect startle in either rat strain. DPAT alone (DPAT/SAL) significantly increased startle amplitude $^{**}p = .01$. The combination of DPAT and CRF (DPAT/CRF) had a greater effect on startle amplitude than DPAT alone $++\ p < .02$ *vs.* DPAT/SAL.

of CRF appears only in rats that were also treated with DPAT, as revealed by a significant IP × ICV interaction, $F(1, 64) = 6.57$, $p < .02$. Thus, a dose of CRF which alone, has no effect on startle amplitude enhanced the effect of DPAT on startle.

Given that the combination of DPAT and CRF both decreased percent PPI and increased baseline startle we sought to assess whether the decrease in PPI was dependent on an increase in startle amplitude. To do this, we performed a median split on the startle amplitude data so that we could examine whether PPI was reduced in both the rats of the DPAT/CRF group that did not show an increase in baseline startle as well as in those that did show an increase in baseline startle. Fig. 3A shows baseline startle amplitude in controls (SAL/SAL) as well as in rats that were treated with DPAT/CRF which did not show an increase in startle amplitude (Low DPAT/CRF) and in those that did show an increase in baseline startle (High DPAT/CRF). As seen in Fig. 3B, both of the DPAT/CRF-treated sub-groups showed a decrease in percent PPI. Here, there was a significant effect of rat strain, $F(1, 30) = 4.5$, $p < .05$. There was also a significant effect of treatment group, $F(2, 30) = 11.5$, $p < .001$, and Tukey post-hoc tests revealed that both the Low DPAT/CRF group ($p = .002$) and the High DPAT/CRF group ($p = .001$) showing less PPI than the SAL/SAL group. There was no strain × group interaction. Thus, the decrease in percent PPI was not caused by the increase in baseline startle.

The effects of rat strain and of treatments on percent habituation of the startle response is shown in Table 1. There was no main effect of rat strain ($p > .05$), IP injection (SAL *vs.* DPAT), $p > .05$, or of ICV infusion (SAL *vs.* CRF), $p > .05$. There was a significant rat strain X ICV infusion interaction, $F(1, 64) = 4.87$, $p < .05$. There was also a significant IP × ICV interaction, $F(1, 64) = 4.61$, $p < .05$, which appears to be due to BN rats although there was no 3-way interaction involving rat strain: DPAT decreased percent habituation in BN rats that received SAL (ICV) but not in those that received CRF (ICV).

3.2. Experiment 2: Effect of IP injection of the 5-HT$_{1A}$ receptor antagonist, WAY, on CRF-Induced changes in startle and PPI

The effects of rat strain, IP injection of WAY and ICV infusion of CRF on percent PPI are shown in Table 2A. An overall ANOVA using these three between-subjects factors was conducted, as well as prepulse stimulus intensity as a within-subjects factor was

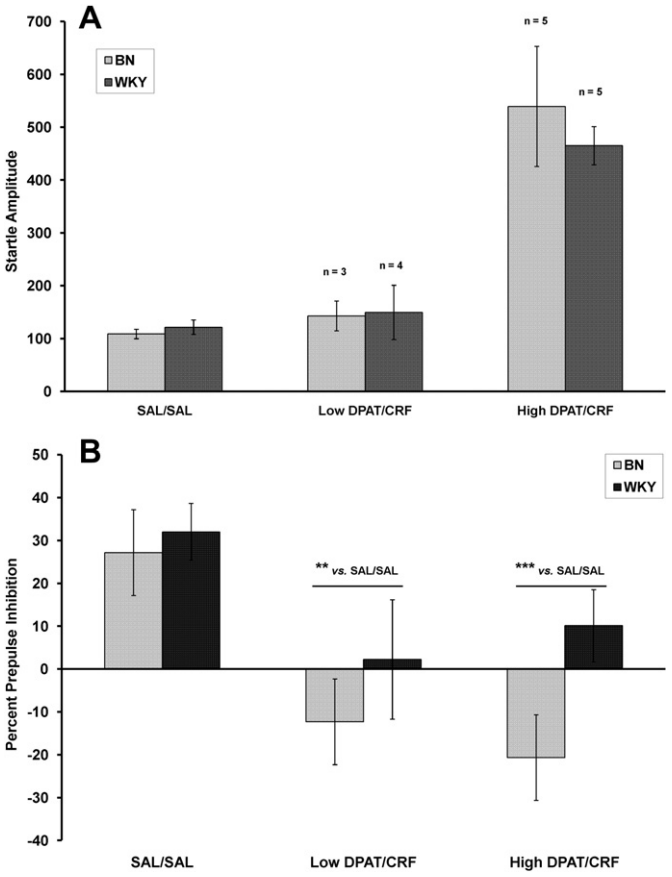

Fig. 3. Startle amplitude (Fig. 3A) and percent PPI averaged across all prepulse stimulus intensities (Fig. 3 B) in rats in which the combination of DPAT and CRF not increase startle amplitude (Low DPAT/CRF) and in those in which startle amplitude was increased by the combined treatment. In Fig. 3B, it can be seen that percent PPI was reduced by the combined treatment whether baseline startle was increased or not. $^{**}p = .002$ *vs.* SAL/SAL; $^{***}p = .001$ *vs.* SAL/SAL.

performed. The results revealed that there was a significant effect of rat strain, $F\ (1,\ 69) = 41.4$, $p < .001$. There was no effect of IP injection of WAY ($p > .05$). There was both a trend towards an effect of ICV infusion of CRF, $F\ (1, 69) = 3.21$, $p = .077$ and towards a rat strain X ICV infusion interaction, $F\ (1, 69) = 3.24$, $p = .076$. There was a significant effect of prepulse stimulus intensity, $F\ (4, 276) = 92.0$, $p < .001$. A significant prepulse intensity X rat strain interaction, $F\ (4, 276) = 16.3$, $p < .001$ with WKY rats being more sensitive to the effect of prepulse stimulus intensity than BN rats. There was also a significant prepulse intensity X rat strain X IP injection interaction, $F\ (4, 276) = 3.1$, $p < .02$. This complex interaction appears to be due to the fact that prepulse intensity had less of an effect in BN rats treated with WAY than in those treated with SAL and that this effect on the response to prepulse intensity was not as great in WKY rats.

Table 1
The effects of CRF and DPAT on percent habituation (mean ± SEM) of the startle response in BN and WKY rats. Neither treatment alone, nor the combination of the treatments affected within-session habituation. Percent habituation was calculated as 100−100*(average startle amplitude on the last 6 startle alone trials/average startle amplitude on the first 6 startle alone trials).

	BN Rats	WKY Rats
SAL/SAL	44.9 ± 9.0	58.7 ± 4.2
SAL/.3 μg CRF	53.0 ± 6.9	40.5 ± 9.9
DPAT/SAL	34.1 ± 8.2	54.9 ± 4.7
DPAT/.3 μg CRF	61.4 ± 8.7	62.8 ± 5.2

Table 2B shows the effect of CRF and WAY, alone and in combination of the amplitude of the baseline startle response in both rat strains. IP injection, ICV infusion and rat strain were between-subjects factors. There was a significant effect of IP injection, $F\ (1, 69) = 22.4$, $p < .001$, with WAY causing a reduction in startle amplitude. There was also a trend for CRF to decrease startle amplitude, $F\ (1,\ 69) = 3.6$, $p = .062$. There were no interactions. Using the same factors, the percent habituation data were also subjected to a 3-way ANOVA (Table 2 C). The results revealed a significant main effect of rat strain, $F\ (1, 69) = 18.7$, $p < .001$ with BN rats showing less habituation than WKY rats. There were no other main effects or interactions.

4. Discussion

CRF (.3 μg) reduced PPI in BN, but not in WKY rats as we have previously shown (Conti, 2005). The 5-HT_{1A} receptor agonist, DPAT, cause a small, but not significant reduction in PPI when administered in the absence of CRF, and added to the effect of CRF on PPI in BN rats. In fact, when the CRF and DPAT were used in combination in this rat strain, prepulse facilitation was found. While neither CRF nor DPAT alone affected PPI in WKY rats, the combined treatment significantly reduced PPI. Thus, sub-threshold doses of CRF and DPAT appear to act synergistically to reduce PPI in WKY rats. However, since only a single dose of DPAT was used and since the effect of the combined DPAT/CRF treatment was not significantly different than the effect CRF alone, it is difficult to be sure that the two treatments resulted in a synergistic effect. Although there was an overall significant main effect of DPAT on PPI (reduction in PPI), it is unclear why DPAT alone failed to significantly reduce PPI in this experiment when data from each rat strain were examined separately. Others have shown that DPAT does result in such a reduction (Gogos and van den Busse, 2007; Rigdon and Weatherspoon, 1992), although some find that this effect is only seen in rats with high baseline levels of PPI (Gogos and van den Busse, 2007). Nevertheless, an effect of DPAT on PPI was revealed when the agonist was administered to CRF-treated rats of either rat strain. Thus, CRF effects may interact with 5-HT_{1A} agonist effects to reduce PPI. The results of this study also show that the effect of the combined treatment with CRF and DPAT on PPI were not due to alterations in baseline startle amplitude.

CRF alone did not alter baseline startle amplitude in either rat strain while DPAT alone caused a small, but significant increase in baseline startle amplitude. However, when administered with the non-effective dose of CRF, DPAT resulted in a larger increase in startle amplitude than it did when administered alone. Others have shown that nicotine withdrawal-induced enhancement of baseline startle is potentiated DPAT (Rasmussen et al., 1997). Given the results of the present study, it would be interesting to know whether this effect of withdrawal is mediated by CRF. Both CRF and the 5-HT_{1A} receptor system have been individually implicated in stress-related anxiety disorders. However, both animal studies (Heisler et al., 1998; Parks et al., 1998; Ramboz et al., 1998) and clinical studies suggest that reductions in the 5-HT_{1A} receptor contribute to the disorders (Lanzenberger et al., 2007; Nash et al., 2008). Thus, this synergistic effect to increase startle amplitude was unexpected. This study is the first in which the combined treatment with CRF and a 5-HT_{1A} receptor agonist on either startle or PPI was examined. The results suggest that further studies of the interactions between these two agonists on anxiety-like behavior are warranted.

Given the results of studies showing that 5-HT_{1A} receptor null mice show more anxiety-like behavior that WT controls (Heisler et al., 1998; Parks et al., 1998; Ramboz et al., 1998), one might have expected that blockade of the receptor with WAY, would

Table 2
The effects of the 5-HT_{1A} antagonist, WAY (IP) and CRF (ICV) alone or in combination on percent prepulse inhibition (2A), baseline startle amplitude (2B) and percent habituation of the startle response (2C). **A**. Percent prepulse inhibition (mean ± SEM) at each of five prepulse stimulus intensities (3–18 dB above background) in BN rats and WKY rats. Rats received an IP injection of either saline (SAL) or the 5-HT_{1A} receptor antagonist, WAY, followed 10 min later by an ICV infusion of either SAL or CRF. The results of all analyses are reported in the text. In BN rats, CRF alone significantly reduced PPI. This effect was not altered by administration of WAY. WAY alone had no effect on PPI. In WKY rats neither treatment alone, nor the combination of treatments affected PPI. **B**. Baseline startle amplitude (mean ± SEM) in BN and WKY rats on the trials from which the data used to calculate percent PPI were collected. Overall ANOVA (see text) revealed that WAY caused a significant reduction in baseline startle amplitude (**$p < .001$). This effect was not rat strain-dependent, and it did not interact with CRF. **C**. The effect of CRF and WAY on percent habituation (mean ± SEM) in BN and WKY rats. Neither treatment alone, nor the combination of treatments affected within-session habituation. Percent habituation was calculated as 100–100*(average startle amplitude on the last 6 startle alone trials/average startle amplitude on the first 6 startle alone trials).

	3 dB PP	6 dB PP	12 dB PP	15 dB PP	18 dB PP
BN Rats					
SAL/SAL	3.8 ± 2.7	5.3 ± 3.3	18.6 ± 2.6	24.9 ± 4.0	36.9 ± 3.4
SAL/.3 μg CRF	−2.6 ± 5.6	−10.2 ± 7.3	10.2 ± 5.9	13.5 ± 5.8	20.5 ± 4.5
WAY/SAL	1.1 ± 3.2	−0.8 ± 5.6	9.1 ± 4.6	18.2 ± 5.2	29.8 ± 4.0
WAY/.3 μg CRF	−1.0 ± 3.7	−15.8 ± 7.3	−2.4 ± 9.9	2.4 ± 11.0	11.7 ± 6.0
WKY Rats					
SAL/SAL	6.8 ± 5.4	18.2 ± 13.5	38.0 ± 5.2	47.6 ± 5.3	56.0 ± 4.9
SAL/.3 μg CRF	3.2 ± 4.3	8.0 ± 7.6	38.8 ± 10.3	44.7 ± 6.5	56.1 ± 5.4
WAY/SAL	−5.3 ± 7.1	−11.0 ± 10.3	44.5 ± 5.6	46.6 ± 5.4	60.4 ± 6.5
WAY/.3 μg CRF	−5.2 ± 13.7	2.5 ± 11.8	49.2 ± 4.4	43.5 ± 9.2	61.4 ± 4.6

B

	BN Rats	WKY Rats	
SAL/SAL	144.3 ± 20.6	120.9 ± 9.9	
SAL/.3 μg CRF	101.4 ± 9.6	124.8 ± 15.9	
WAY/SAL	97.1 ± 9.6	75.6 ± 15.5	**
WAY/.3 μg CRF	81.7 ± 10.0	58.4 ± 11.2	

C

	BN Rats	WKY Rats
SAL/SAL	14.1 ± 8.1	48.1 ± 9.4
SAL/.3 μg CRF	29.7 ± 10.9	53.0 ± 4.0
WAY/SAL	30.6 ± 11.0	60.0 ± 6.5
WAY/.3 μg CRF	36.9 ± 8.1	54.6 ± 6.8

potentiate the effect of CRF on PPI. This did not occur. It should be noted that the dose of WAY used in the present experiments does attenuate behavioral effects caused by DPAT (De Vry et al., 2004). Additionally, WAY alone had no effect on PPI. Yohimbine-induced decreases in PPI are actually blocked by WAY (Powell et al., 2005). Yet, others have shown that WAY does not attenuate the apomorphine-induced decrease in PPI, while it does block the apomorphine-induced increase in baseline startle amplitude (Gogos et al., 2010). Additionally, the nicotine withdrawal-induced increase in startle amplitude is not blocked by WAY (Rasmussen et al., 1997). Thus, blockade of the 5-HT_{1A} receptor appears to diminish the reduction in PPI caused by some treatments, but not by all treatments. Given the effect of 5-HT_{1A} receptor gene knockout on anxiety-like behaviors, it might also have been expected that WAY would reduce baseline startle amplitude. This, in fact, was the case in the present studies.

Here, we show that a 5-HT_{1A} receptor agonist can potentiate the effect of CRF on PPI and startle, while administration of a 5-HT_{1A} receptor antagonist is not sufficient to block the effects of CRF on these behaviors. This may be due to the fact that it is more likely that CRF affects serotonergic neurotransmission rather than that 5-HT affects CRF transmission. Thus, what our results might suggest is that CRF actually potentiates an effect of 5-HT at the 5-HT_{1A} receptor, so that a sub-threshold dose of a 5-HT_{1A} receptor agonist, such as that used here, becomes effective and then adds to the effect of CRF on PPI and startle amplitude. When the 5-HT_{1A} is blocked, CRF remains free to affect PPI independently of 5-HT. Other studies have been conducted in order to explore the potential interactions between the effects of CRF and other neurotransmitters systems on PPI and startle. We have shown that neither serotonin depletion nor administration of the serotonin (5-HT) 5-$HT_{2A/2C}$ receptor antagonist, ketanserin attenuate the effect of CRF on PPI (Sutherland et al., 2008). However, ketanserin does block the CRF-induced increase in baseline startle amplitude and serotonin depletion enhanced the CRF-induced increase in startle (Sutherland et al., 2008). Others have shown that neither deletion of the dopamine D_1 or D_2 receptors, nor administration of antagonists for these receptors affect CRF-induced changes in startle or PPI (Vinkers et al., 2007). The roles of norepinephrine receptors in CRF-induces changes in startle and PPI have also been examined. In those studies, Gresack and Risbrough (2010) found that both the α2-adrenergic receptor agonist, clonidine, and the α1-adrenergic receptor antagonist, prazosin, blocked the CRF-induced increase in baseline startle amplitude, but neither drug affected the CRF-induced decrease in PPI. The β-adrenergic receptor antagonist, propranolol, had no did not affect either the CRF-induced change in startle or PPI. Thus, it appears that while the effect of CRF on PPI and startle can be potentiated by activation of 5-HT_{1A} receptors, CRF can also act independently to reduce PPI.

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