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Both corticotropin-releasing factor and apomorphine reduce prepulse inhibition following repeated central infusion of corticotropin-releasing factor

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Abstract

The neuropeptide, corticotropin-releasing factor (CRF) has been shown to disrupt prepulse inhibition of the acoustic startle response in rodents. Prepulse inhibition is deficient in a number of psychiatric disorders. In Experiment 1, we examined whether repeated central infusion of CRF alters the reduction in prepulse inhibition caused by subsequent CRF infusion or apomorphine injection. Repeated intracerebroventricular infusion of CRF (0.3 μg) did not cause tolerance to the effect of CRF on prepulse inhibition. Additionally, repeated CRF did not alter the effect of apomorphine (0.25 mg/kg, i.p.) on prepulse inhibition. In contrast to other reported results, both CRF and apomorphine reduced baseline startle amplitude in the Brown Norway rats, which show low prepulse inhibition. In Experiment 2, we showed that a CRF-induced change in baseline startle amplitude does not contribute to the CRF-induced decrease in percent prepulse inhibition. In Experiment 3, we found that methylphenidate (20.0 mg/kg, i.p.) increased baseline startle amplitude in Brown Norway rats, yet it also decreased percent prepulse inhibition. These results suggest that CRF can be administered repeatedly without diminution of its effects on prepulse inhibition, and that in Brown Norway rats, compounds that either increase or decrease baseline startle amplitude can reduce percent prepulse inhibition independently of the effects on baseline startle.

Keywords: Apomorphine; Brown Norway rats; CRF; Methylphenidate; Prepulse inhibition; Sensorimotor gating; Startle

1. Introduction

Sensorimotor gating deficits, as assessed by prepulse inhibition (PPI) of the startle response, are found in patients with schizophrenia (Braff et al., 1978; Geyer et al., 1990; Mackeprang et al., 2002; Parwani et al., 2000). PPI is the reduction in startle amplitude that results from brief presentation of a non-startling stimulus, shortly prior to presentation of a startling stimulus (Hammond et al., 1972). PPI can be established and assessed in both humans and rodents under very similar circumstances, making it a useful endophenotype to study the neurobiology and pharmacology of psychiatric disorders. While direct dopamine (DA) receptor agonists, such as apomorphine, can disrupt PPI in rats (Geyer et al., 1990; Mansbach et al., 1988), the effect is rat strain-dependent, such that some strains show less sensitivity to the PPI-disrupting effect of apomorphine than others, and some strains lack sensitivity to apomorphine in this test (Rigdon, 1990; Swerdlow et al., 2001, 2004, 2005).

We and others have begun to examine and characterize a role for the neuropeptide, corticotropin-releasing factor (CRF) in the expression of PPI. CRF is a hypothalamic peptide that is released as a hormone during stress (Rivier and Vale, 1983), and numerous lines of evidence suggest that extra-hypothalamic CRF also acts as a neurotransmitter in a number of brain regions with resulting behavioral effects (see Bale and Vale, 2004 for review; Gabr et al., 1991; Van Bockstaele et al., 1998). CRF receptors are expressed

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in the cortex, striatum, hippocampus, nucleus accumbens (NAc) and the basolateral nucleus of the amygdala (Chalmers et al., 1995; De Souza, 1987; Radulovic et al., 1998), areas that are important for control of PPI (Bakshi and Geyer, 1999; Swerdlow et al., 1994). Intracerebroventricular (i.c.v.) infusion of CRF to either rats (Conti et al., 2002, 2005; Conti, 2005; Hammer and Arrillaga-Romany, 2004) or mice (Risbrough et al., 2004) reduces PPI of the acoustic startle response. This effect of CRF is long-lasting (Bakshi et al., 2004) and is attenuated by antipsychotic drugs (Conti et al., 2005; Hammer and Arrillaga-Romany, 2004). Additionally, transgenic mice that over-express CRF show diminished PPI compared to wild type (WT) mice and antipsychotic drugs enhance PPI in the transgenic strain (Dirks et al., 2003). While CRF remains anxiogenic following repeated infusion (Song et al., 1995), a single or repeated pretreatment causes long-lasting desensitization of locus coeruleus electrophysiological responsivity to subsequent CRF (Conti and Foote, 1995). It has been hypothesized that behavioral dysfunction associated with repeated/prolonged stress may involve alterations in CRF neurotransmission, as well as alterations in sensitivity to both CRF itself, and to drugs that directly affect other neurotransmitter systems. Although CRF over-expressing mice display reduced PPI (Dirks et al., 2002), it is not known whether exogenous CRF continues to reduce PPI after repeated central infusion or whether tolerance develops.

CRF (i.c.v.) also increases both DA utilization and DA concentrations in frontal cortex, nucleus accumbens, hippocampus and amygdala (Kalivas et al., 1987; Lavicky and Dunn, 1993; Matsuzaki et al., 1989), and other lines of evidence suggest that CRF and DA have interactions with behavioral consequences. For example, the startle-enhancing effect of CRF is attenuated by a selective DA D_1 receptor antagonist (Meloni et al., 2006). Additionally, repeated central infusion of CRF has been shown to alter behavioral sensitivity to the indirect DA receptor agonist, amphetamine (Cador et al., 1993; Cole and Koob, 1989; Izzo et al., 2005).

In the present experiments, we examined whether repeated i.c.v. infusion of CRF would alter the response to a subsequent infusion of CRF on PPI and baseline startle. We also examined whether repeated central infusion of CRF would alter the reduction in PPI caused by the DA receptor agonist, apomorphine. Additionally, these experiments allowed us to examine whether acute apomorphine diminishes PPI in Brown Norway (BN) rats, a strain in which the effects of apomorphine have yet to be examined.

CRF does not enhance baseline startle amplitude in BN rats, as it does in other strains, (Conti, 2005; Liang et al., 1992; Swerdlow et al., 1989). In fact, the present results suggest that that acute infusion of CRF actually decreases startle amplitude in BN rats, an effect that has not been reported in other rat strains. However, CRF over-expressing mice, which show diminished PPI, do show lower baseline startle amplitude than WT controls (Dirks et al., 2002). Therefore, we examined whether CRF also reduces PPI under conditions in which startle amplitude in CRF-treated rats is equivalent to startle amplitude in saline-treated rats. This was achieved by using two startle stimulus intensities in the same testing session.

Finally, given the effects of CRF on baseline startle in the BN strain, we examined whether PPI could be disrupted in this strain by a dose of methylphenidate which increases, rather than decreases, startle amplitude. This was done to examine whether PPI is only reduced in BN rats by treatments which decrease baseline startle amplitude. In this experiment, both BN rats and Wistar–Kyoto (WKY) rats were used, as we have previously found that BN rats are more sensitive to CRF-induced disruption of PPI than WKY rats (Conti, 2005). While methylphenidate has been shown to disrupt PPI in Wistar rats, it has been shown not to do so in WKY rats (Drolet et al., 2002). We used both a low, clinically relevant dose of methylphenidate and a high dose, which has behavioral effects that are comparable to those produced by doses of amphetamine that disrupt PPI (Kuczenski and Segal, 1997; Swerdlow et al., 2003a).

2. Methods and materials

2.1. Experimental animals

For all experiments, male Brown Norway rats (Harlan Sprague Dawley) were obtained at 11–12 weeks of age. For Experiment 3, Wistar–Kyoto (WKY) rats (Charles River) were also used. Rats were double-housed and allowed to acclimate to our colony, which is on a 12 h light/12 h dark cycle, for 7–9 days prior to surgery. Laboratory chow and water were available ad libitum. All procedures were done in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals, and the experimental protocols were approved by an Institution Review Committee for the Care and Use of Animals.

2.2. Surgery to implant i.c.v. guide cannula

Rats were anesthetized with a mixture of isoflurane-in-oxygen (1.5%) and placed into a stereotaxic instrument equipped with blunt ear bars. A stainless-steel guide cannula (22-gauge) was aimed at the lateral ventricle (1.0 mm posterior and 2.0 mm lateral to Bregma) for subsequent intracerebroventricular (i.c.v.) infusion of either saline or rat/human CRF. Two jewelers' screws were placed into the skull, and the entire assembly was held in place with dental cement. A dummy cannula was then placed into the guide. Cannula placements were assessed in random animals by infusion of methylene blue dye and verification of dye in the ventricular system.

2.3. Apparatus and testing

For testing, rats were placed into a startle testing chamber consisting of a clear acrylic cylindrical chamber (10 cm diameter, 14 cm length) that is enclosed in a sound- and vibration-attenuating cabinet. The cabinet is equipped with a 20 W incandescent bulb and a fan for ventilation (San Diego Instruments). The chamber sits upon a base, under which is a piezoelectric accelerometer which detects whole body startle responses. Output signals from the accelerometer were collected as 100 sequential 1 ms measurements starting at the onset of the

startling stimulus. Signals from the accelerometer were rectified, digitized and stored on computer by SR-LAB program (San Diego Instruments). Chambers were calibrated each day and matched for sound intensity. Delivery of white noise acoustic stimuli, through a horn tweeter (Radio Shack), was also controlled by the SR-LAB program.

Testing was conducted as previously described (Conti et al., 2005). A 5-min acclimation period, during which only the background noise (70 dB) was presented occurred prior to the delivery of any stimulus. All stimuli were presented on this background noise. In Experiment 1, there were 82 trials in the testing session and, on the first and last six trials, the startling stimulus (white noise, 50 dB above background = 120 dB total, 40 ms) was presented alone. The remaining trials included additional startle stimulus alone trials (used to calculate percent PPI) and trials on which a prepulse stimulus of which 3, 6, 12, 15 or 18 dB above background (20 ms duration, 10 trials with each prepulse intensity) preceded the startling stimulus by 100 ms. Additionally, there were eight trials on which no stimulus was presented, but activity within the testing chamber was assessed. These trials were presented in pseudorandom order, and the inter-trial interval averaged 20 s (15–25 s). In Experiment 2, two startle stimulus intensities were used in the same testing session: One was 35 dB above background (105 dB total) and the other was 50 dB above background (120 dB total). Both were 40 ms in duration and both occurred 100 ms after prepulse stimuli, which were 6, 12, 15 and 18 dB above background. In Experiment 3, only the 120 dB startling stimulus was used, and the prepulse stimuli were 3, 6 and 12 dB above background. Experiment 3 was conducted while the first author was at the University of California, San Diego. Thus, while the testing equipment was also from San Diego Instruments, the environment was not the same as that in Experiments 1 and 2. However, the BN rats were from the same vendor. The inter-trial interval in all experiments averaged 15 s. All testing took place between the hours of 10:00 a.m. and 3:00 p.m.

2.4. Experimental procedures

2.4.1. Experiment 1

One week after surgery, rats were put on a course of repeated i.c.v. infusions. Half the rats received infusions of saline and the other half received infusions of CRF (0.3 μg in 6.0 μl). Rats were infused once a day for 4 consecutive days. On day 5, test day, rats were brought from the colony to the laboratory, and the groups were further sub-divided such that rats from each repeated treatment group received either: (1) an injection of saline (i.p.); (2) an i.c.v. infusion of CRF (0.3 μg); (3) an injection of apomorphine (0.25 mg/kg, i.p.). No group received i.c.v. saline on day 5. We have collected extensive data on PPI following acute i.c.v. saline (Conti et al., 2002, 2005; Conti, 2005) and found that, 30 min following i.c.v. saline, PPI is extremely comparable to the level seen in 30 min after an i.p. injection of saline seen in the present study. Thus, we controlled for the apomorphine injection rather than the final i.c.v. infusion. The design of this experiment is outlined below.

Repeated treatment	Treatment on day 5 (test day)	Group name (*n*)
Saline (i.c.v.)	Saline (i.p.)	SAL/SAL (10)
Saline (i.c.v.)	CRF (i.c.v.)	SAL/CRF (10)
Saline (i.c.v.)	Apomorphine (i.p.)	SAL/APO (10)
CRF (i.c.v.)	Saline (i.p.)	CRF/SAL (9)
CRF (i.c.v.)	CRF (i.c.v.)	CRF/CRF (10)
CRF (i.c.v.)	Apomorphine (i.p.)	CRF/APO (11)

Rats were tested for baseline startle amplitude and PPI of the startle response beginning 10 min after the apomorphine injection or 30 min after the CRF infusion.

2.4.2. Experiment 2

In Experiment 1, CRF decreased baseline startle amplitude. In this experiment, we sought to equalize the baseline startle amplitude in saline- and CRF-treated rats. This was done to address the possibility that CRF only alters percent prepulse inhibition because it alters baseline startle amplitude (Davis, 1988; Risbrough et al., 2004). Therefore, we tested rats according to a method employed by Risbrough et al. (2004) such that both saline- and CRF-treated rats (saline, $n=7$; CRF, $n=8$) were tested in a session in which both the 120 dB startling stimulus, and a 105 dB startling stimulus were used. This was done so that baseline startle amplitude in response to the 105 dB starling stimulus in saline-treated rats would be equivalent to baseline startle amplitude in response to the 120 dB startling stimulus in CRF-treated rats. An equivalent number of prepulse stimuli (either 6, 12, 15 or 18 dB above background), preceded an equivalent number of low- and high-intensity startling stimuli.

2.4.3. Experiment 3

In this experiment, we examined the effect of the indirect DA agonist, methylphenidate on startle amplitude and PPI. Rats did not undergo surgery for this experiment. BN and WKY rats were injected with either saline, or one of two doses of methylphenidate (1.0 or 20.0 mg/kg, i.p.) immediately before being tested for PPI and startle amplitude. There were 10 WKY rats/group, 10 BN rats in the saline group and 11 BN rats in each of the two methylphenidate groups.

2.5. Data analysis

Percent PPI was calculated for each rat at each prepulse stimulus intensity as follows: percent PPI = 100 − (100 × [prepulse/startle]). Prepulse was the average startle amplitude on trials in which a prepulse stimulus preceded the startling stimulus, and startle was the average amplitude on trials in which the startling stimulus was presented alone. Data from the first and last six trials were not used in this calculation. Average startle amplitude on the startle alone trials used to calculate percent PPI was also calculated.

For Experiment 1, percent PPI data were subjected to two analyses of variance (ANOVAs); one using the data from rats that were treated with CRF on test day and a second using the data from rats that were treated with apomorphine on the test day. Both were three-way ANOVAs with repeated treatment and test day treatment as between-subjects factors, and prepulse stimulus intensity as a within-subjects factor. When

significant CRF treatment effects were found, additional ANOVAs were conducted to compare the effect of test day CRF or apomorphine to test day saline. For this purpose, ANOVAs were conducted for the repeated saline-treated and repeated CRF-treated groups. Baseline startle data (from trials on which the startling stimulus was presented alone) were analyzed with two-way ANOVAs with repeated treatment and test day treatment as between-subjects factors. Again, when significant treatment effects were found, additional ANOVAs were conducted to compare the effect of test day CRF or apomorphine to test day saline. Separate ANOVAs were conducted for the repeated saline-treated and repeated CRF-treated groups.

For Experiment 2, percent PPI data were subjected to a three-way ANOVA with infusate (saline vs. CRF) as a between-subjects factor, and startle stimulus and prepulse stimulus intensities as within-subjects factors. We also examined whether there was a difference between the CRF- and saline-treated groups in percent PPI at the 105 and 120 dB stimulus conditions separately. Baseline startle amplitude data were subjected to a

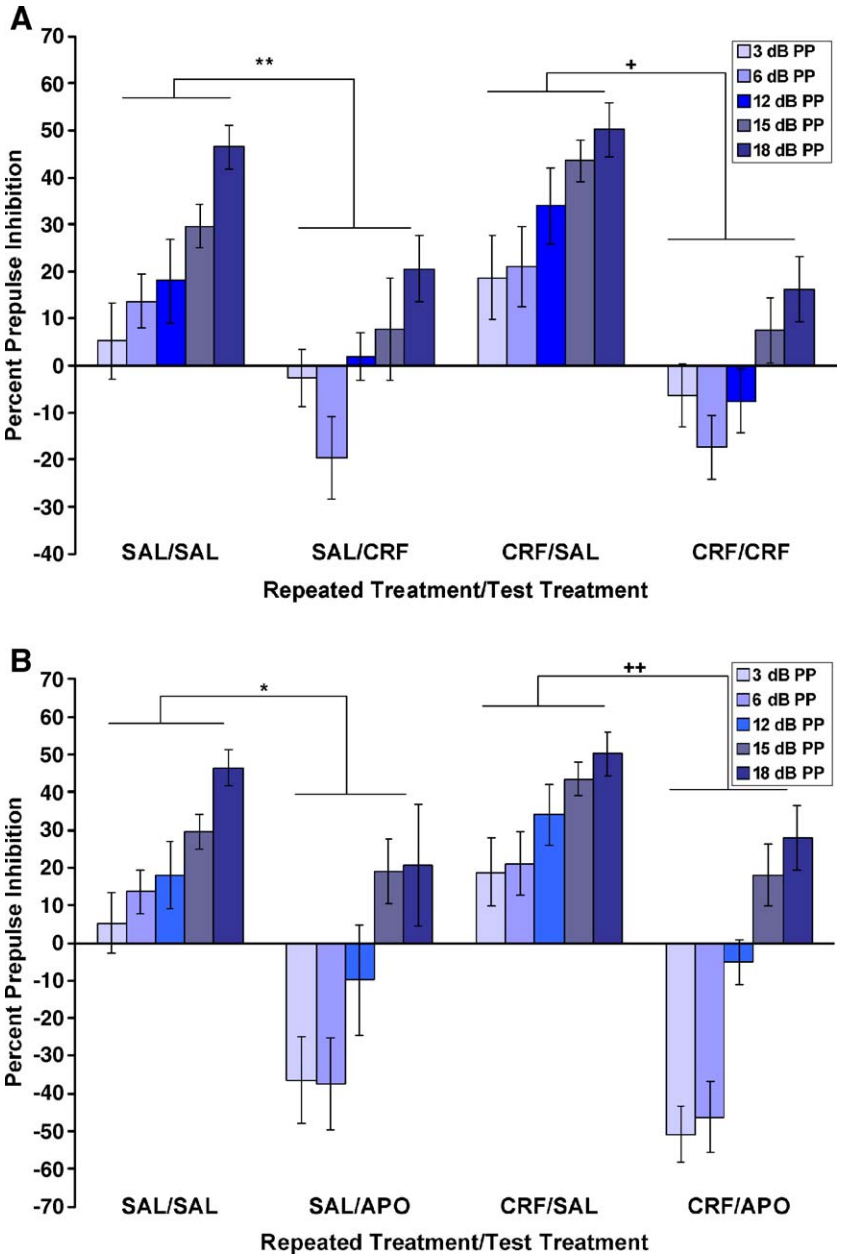

Fig. 1. Percent prepulse inhibition (mean ± S.E.M.) in rats that received repeated infusion of either saline or CRF, and subsequently received either saline (SAL, i.p.) or CRF on test day (A), or received SAL or apomorphine (APO) on test day (B). Repeated CRF alone had no effect on PPI in rats that received saline on test day. CRF treatment on test day produced an equivalent decrease in PPI in both the group that had received repeated saline and the group that has received repeated CRF (A). Similarly, APO treatment on test day resulted in an equivalent decrease in PPI in both the group that had received repeated saline and the group that has received repeated CRF. *$p<0.02$, **$p<0.01$, $^{+}p=0.001$, $^{++}p<0.002$.

two-way ANOVA with infusate as a between-subjects factor and startle stimulus intensity as a within-subjects factor. A dependent *t*-test was used to examine the effect of CRF in the two startle stimulus conditions. Additionally, an independent *t*-test was conducted to examine whether there was a difference in startle amplitude between the saline-treated rats at the 105 dB stimulus and the CRF-treated rats at the 120 dB stimulus. Finally, a dependent *t*-test was conducted to examine whether startle amplitude was affected by startle stimulus intensity in the CRF-treated rats alone.

For Experiment 3, percent PPI data were subjected to a three-way ANOVA with rat strain and methylphenidate dose as a between-subjects factors and prepulse stimulus intensity as a within-subjects factor. This was followed by a Tukey post-hoc test. Baseline startle amplitude data were subjected to the same analysis.

2.6. Peptides and drugs

CRF was kindly provided by Dr. Jean Rivier (Salk Institute). The peptide was dissolved in saline, and aliquots were stored at −80 °C. Apomorphine was purchased from Sigma-Aldrich and dissolved in saline fresh daily. Methylphenidate was a gift from Dr. Ronald Kuzcenski (University of California, San Diego).

3. Results

3.1. Experiment 1

Fig. 1 shows the effect of repeated CRF infusion on the response to CRF infusion test day (Fig. 1A) and on the response to apomorphine on test day (Fig. 1B). In Fig. 1A, it can be seen that there was no significant main effect of repeated CRF treatment, *F*

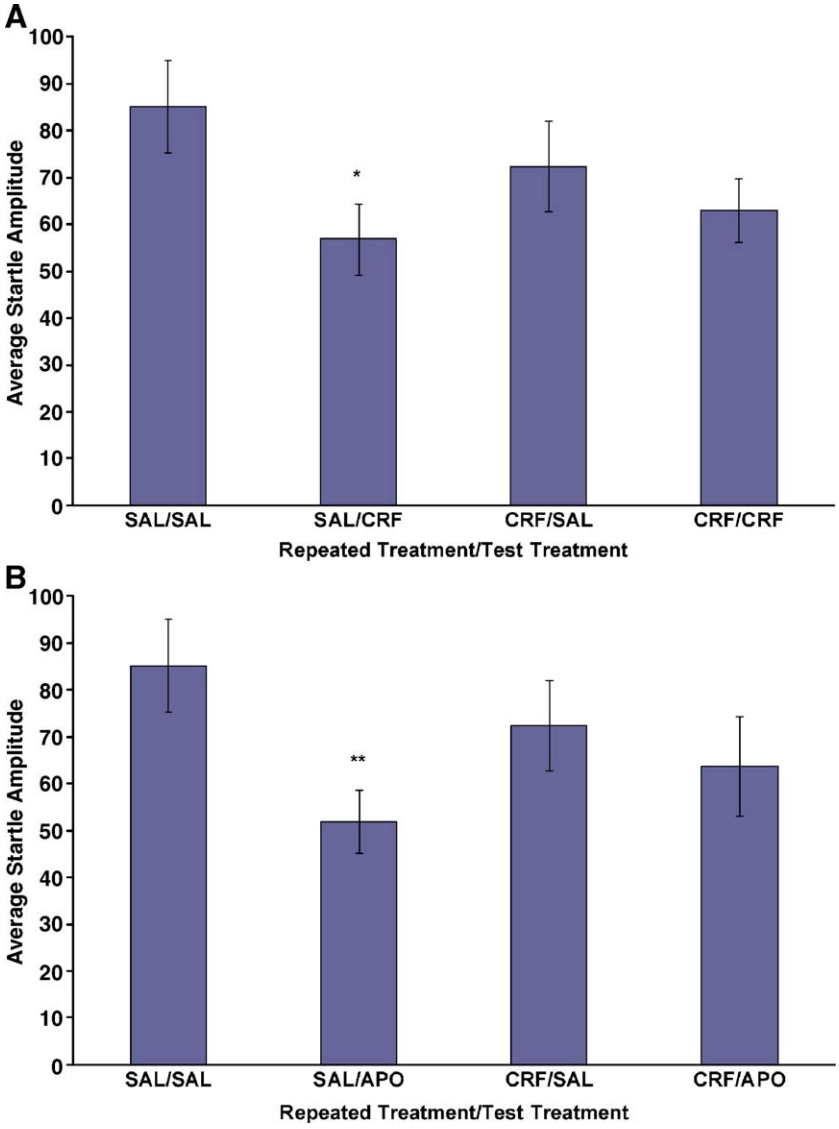

Fig. 2. Mean (±S.E.M.) startle amplitude in response to the startling stimulus alone on the trials that were used to calculate percent PPI. Both CRF (A) and APO (B) caused a significant reduction in startle amplitude whether the rats had received repeated saline or repeated CRF prior to test day. *$p<0.05$ vs. SAL/SAL, **$p<0.02$ vs. SAL/SAL.

(1,35)<1. There was a significant effect of CRF on test day, $F(1,35)=26.8$, $p<0.001$, with CRF reducing percent PPI. However, there was no repeated treatment×test day treatment interaction, $F(1,35)=1.7$, $p>0.05$. As can be seen in Fig. 1A, this lack of an interaction is due to the fact that repeated CRF did not alter the effectiveness of CRF on test day and that repeated CRF had no effect on PPI in rats that were injected with saline on test day. There was a main effect for prepulse stimulus intensity, $F(4,140)=27.1$, $p<0.001$, but no significant interactions involving this repeated measure. Separate ANOVAs revealed that percent PPI was significantly less in the SAL/CRF group than in the SAL/SAL group, $p<0.01$ and was significantly less in the CRF/CRF group than in the CRF/SAL group, $p=0.001$. In Fig. 1B, it can be seen that there was again no significant effect of repeated CRF treatment, $F(1,36)<1$. There was a significant effect of apomorphine treatment on test day, $F(1,36)=30.9$, $p<0.001$, with apomorphine reducing percent PPI. Again, there was no repeated treatment×test day treatment interaction, $F(1,36)<1$. There was a significant main effect for prepulse stimulus intensity, $F(4,144)=47.7$, $p<0.001$. Here, there was also a prepulse stimulus intensity×test day treatment interaction, $F(4,144)=7.6$, $p<0.001$, which can be seen as the prepulse facilitation caused by apomorphine at the low prepulse stimulus intensities. Additionally, percent PPI was significantly less in the SAL/APO group than in the SAL/SAL group ($p<0.02$), and significantly less in the CRF/APO than in the CRF/SAL group ($p<0.001$).

The effects of repeated and test day treatments on baseline startle amplitude are shown in Fig. 2. In Fig. 2A, there was no effect of repeated treatment, $F(1,35)<1$. There was a significant effect of CRF on test day, $F(1,35)=4.9$, $p<0.05$, with CRF

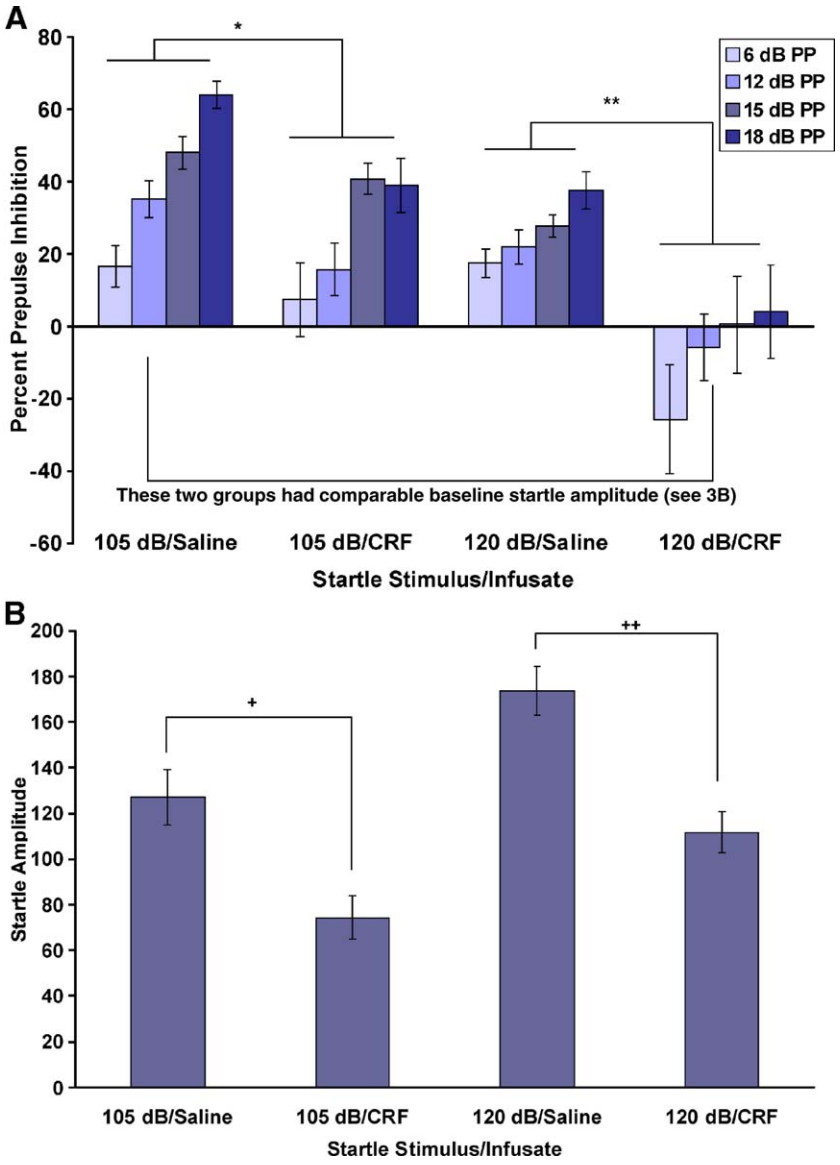

Fig. 3. Percent PPI (mean±S.E.M.) in saline- and CRF-treated rats on trials on which the startling stimulus was 105 dB and on trials on which the startling stimulus was 120 dB is shown in (A). There was a significant effect of CRF on PPI and a significant effect of startle stimulus intensity on PPI. As can be seen in (B), baseline startle amplitude was equivalent in the saline-treated group under the 105 dB condition and the CRF-treated group under the 120 dB condition. This suggests that the effect of CRF on percent PPI is not the result of an altered startle baseline. *$p<0.05$, **$p<0.02$, $^{+}p<0.01$, $^{++}p=0.001$.

reducing startle amplitude. This was due to the difference between the SAL/SAL group and the SAL/CRF group ($p<0.05$). There was repeated treatment × test day treatment interaction, $F(1,35)=1.2$, $p>0.05$, which was due to the fact that CRF on test day did not significantly reduce startle amplitude in rats that were repeatedly treated with CRF compared to their repeated treatment controls (CRF/SAL). In Fig. 2B, there was no main effect of repeated treatment on startle amplitude, $F(1,36)<1$. There was a significant effect of apomorphine on test day, $F(1,36)=4.9$, $p<0.05$, with apomorphine reducing startle amplitude. This was due to a difference between the SAL/SAL group and the SAL/APO group ($p<0.02$). There was no repeated treatment × test day treatment interaction, $F(1,36)=1.7$, $p>0.05$.

3.2. Experiment 2

In this experiment, two startle stimulus intensities were used so that we could examine the effect CRF on PPI under conditions in which baseline startle was equivalent to that seen in saline-treated rats. Fig. 3A shows the effect CRF on percent prepulse inhibition and startle amplitude under the 105 dB and 120 dB startling stimulus intensity conditions. There was a significant overall effect of CRF on percent PPI (Fig. 3A), $F(1,13)=11.6$, $p=0.005$, with lower PPI occurring in the CRF-treated groups than in the saline-treated groups. Additionally, CRF significantly reduced percent PPI at both the 105 dB startling stimulus intensity, $p<0.05$, and at the 120 dB startling stimulus intensity, $p<0.02$. There was also a significant effect of startle stimulus intensity on percent prepulse inhibition, $F(1,39)=20.0$, $p=0.001$, with greater inhibition occurring at the 120 dB startle stimulus intensity, and a significant effect of prepulse stimulus intensity, $F(3,39)=14.9$, $p<0.001$. There were no significant interactions involving either of these within-subjects factor.

Fig. 3B shows the effect of both startle stimulus intensity and CRF on baseline startle amplitude. There was a significant effect of stimulus intensity, $F(1,13)=53.7$, $p<0.001$, with the higher

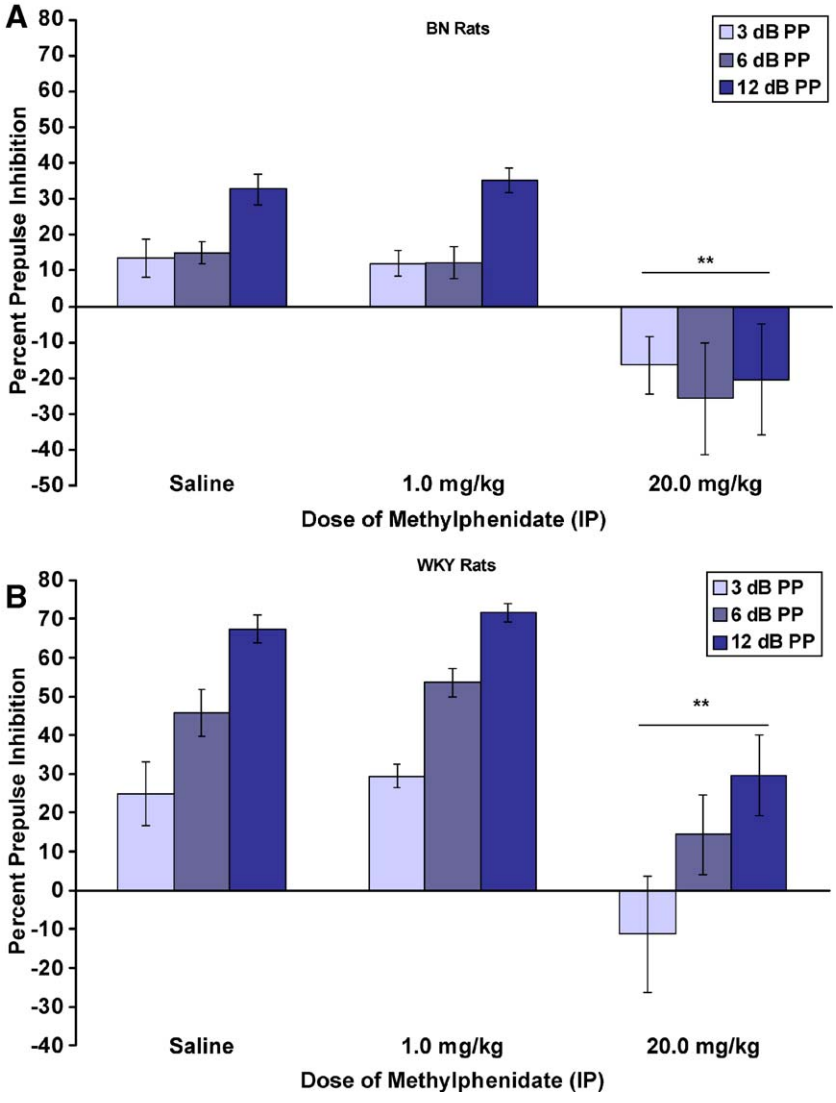

Fig. 4. The effect of methylphenidate on mean (±S.E.M.) percent PPI in BN rats (A) and WKY rats (B). PPI was significantly reduced by 20.0, but not by 1.0 mg/kg methylphenidate. **$p<0.01$ vs. both saline and 1.0 mg/kg.

intensity resulting in higher amplitude, and of CRF, $F(1,13)=18.5$, $p=0.001$, with CRF decreasing startle amplitude. There was no interaction between these two factors. Additionally, CRF reduced startle amplitude at both the 105 dB intensity, $p<0.01$, and at the 120 dB intensity $p=0.001$. An independent *t*-test revealed that there was no significant difference in startle amplitude between the saline-treated group at the 105 dB startle stimulus intensity, and the CRF-treated group at the 120 dB startle stimulus intensity ($t(13)=1.0$, $p>0.05$). However, a dependent *t*-test revealed that there was a significant difference in baseline startle amplitude between the 105 and 120 dB stimulus intensities in the CRF-treated group ($t(7)=4.7$, $p=0.002$.

3.3. Experiment 3

The effect of methylphenidate on PPI in BN and WKY rats is shown in Fig. 4. There was a significant effect of rat strain, $F(1,56)=26.9$, $p<0.001$, with BN rats (Fig. 4A) showing less PPI than WKY rats (Fig. 4B). There was also a significant effect of methylphenidate dose, $F(2,56)=21.4$, $p<0.001$, with methylphenidate reducing percent PPI, but there was no rat strain × dose interaction, $F<1$. There was a significant effect of prepulse stimulus intensity, $F(2,112)=34.7$, $p<0.001$, as well as a prepulse stimulus intensity × rat strain interaction, $F(2,112)=11.6$, $p<0.001$. There were no other significant interactions. Separate ANOVAs revealed that the effect of methylphenidate was

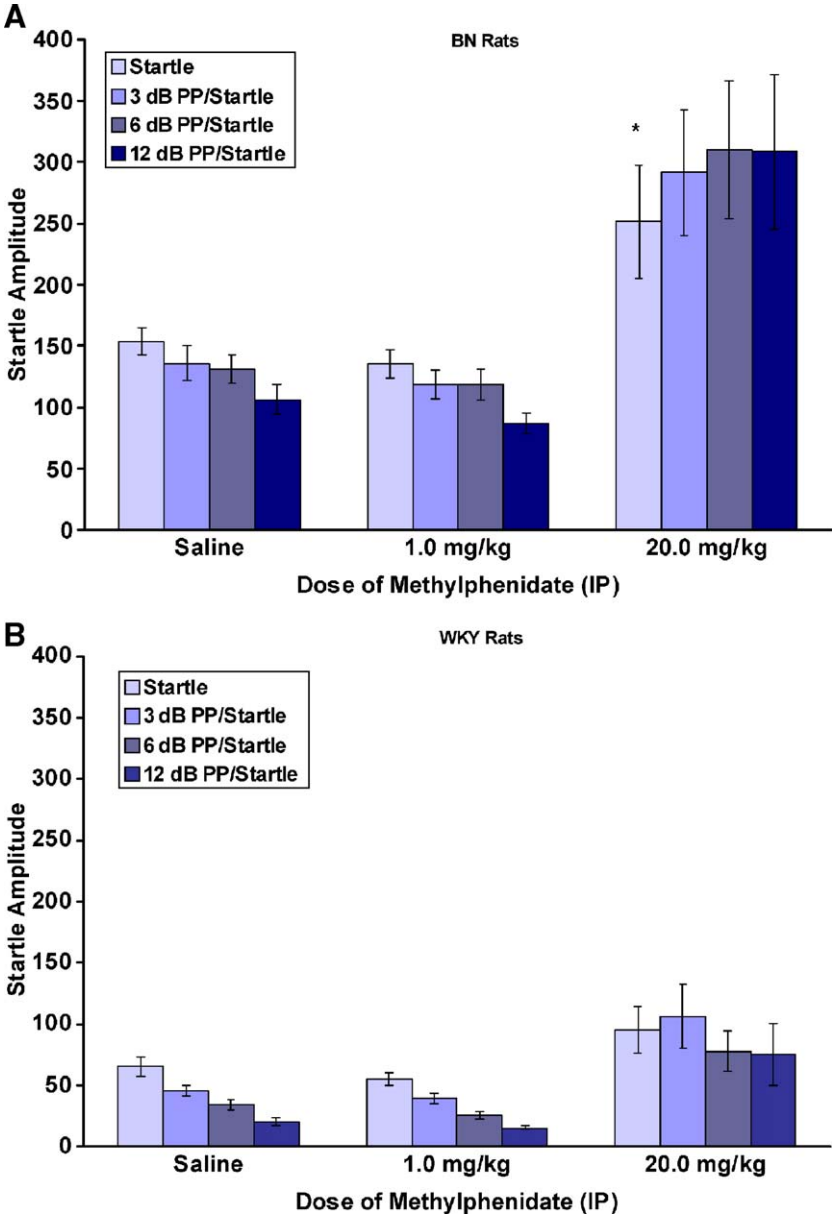

Fig. 5. The effect of methylphenidate on absolute startle amplitude on trials on which the startling stimulus was presented alone, and trials on which prepulse stimuli preceded the startling stimulus. Although 20.0 mg/kg methylphenidate significantly increased baseline startle in BN rats (A) and marginally increased startle amplitude in WKY rats (B), prepulse stimuli did not reduce startle amplitude in these groups. *$p<0.02$ vs. 1.0 mg/kg.

significant in the BN rats alone, $F(2,29)=12.1$, $p<0.001$, with Tukey tests showing that there was significantly less PPI in the 20 mg/kg group than either the saline ($p=0.001$) or the 1.0 mg group ($p=0.001$). Methylphenidate also reduced percent PPI in the WKY rats, $F(2,27)=9.5$, $p=0.001$, and Tukey tests revealed that there was a significant difference between the 20.0 mg/kg dose and saline ($p=0.005$), as well as between the 20.0 mg/kg and 1.0 mg/kg doses ($p=0.001$).

Fig. 5 shows absolute values of startle amplitude in response to the startling stimulus alone, as well as on the trials on which prepulse stimuli were presented. Data on the trials on which the startle stimulus was presented alone were subjected to ANOVA. For this measure, there was a significant effect of rat strain, $F(1,56)=33.9$, $p<0.001$, and of methylphenidate dose, $F(2,56)=6.8$, $p=0.002$, with methylphenidate significantly enhancing startle amplitude. While there was not a significant rat strain × dose interaction, it can be seen that absolute change in startle amplitude caused by the 20.0 mg/kg dose of methylphenidate was greater in BN than in WKY rats. Therefore, two separate two-way ANOVAs were conducted to examine the effect of dose on baseline startle amplitude. In BN rats, there was a significant effect of dose, $F(2,29)=4.8$, $p<0.02$, and Tukey tests showed that, while there was a only a marginal difference between the effect of saline and 20.0 mg/kg methylphenidate ($p=0.062$), there was a significant difference between the effects of 1.0 mg/kg and 20.0 mg/kg ($p<0.02$). The effect of methylphenidate dose on startle amplitude did not reach statistical significance in WKY rats, $F(2,27)=2.9$, $p>0.05$. Fig. 5 also shows that, while 20.0 mg/kg methylphenidate increased baseline startle amplitude in BN rats above that seen in the group treated with 1.0 mg/kg, this effect did

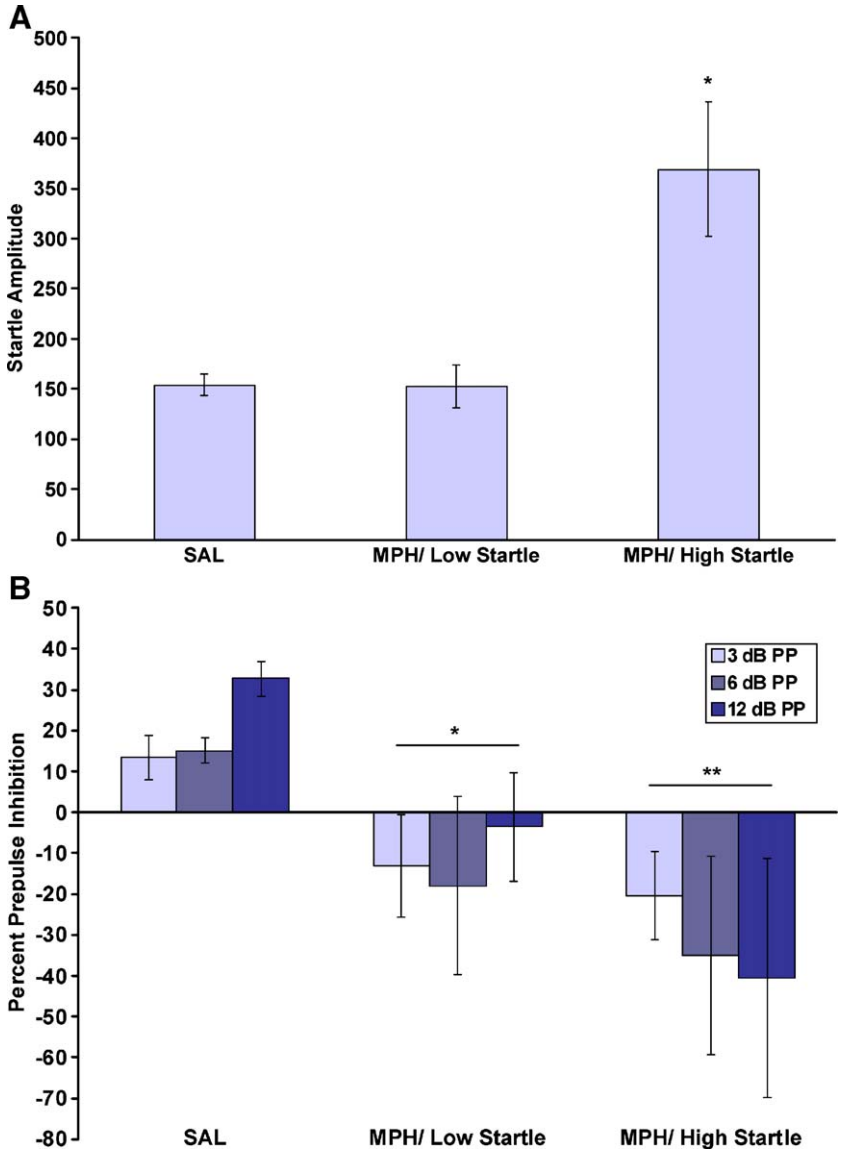

Fig. 6. Mean (±S.E.M.) startle amplitude (A) in saline-treated BN rats and in the BN rats in which the 20.0 mg/kg dose of methylphenidate (MPH) did not affect startle amplitude (low startle, $n=6$) and those in which startle amplitude was increased (high startle, $n=5$). *$p<0.001$ vs. both SAL and low startle. Mean (±S.E.M.) percent prepulse inhibition (B) in the saline-treated group and the two sub-groups of methylphenidate treated rats for which startle amplitude is show in (A). *$p<0.05$ vs. SAL, **$p=0.02$ vs. SAL.

not contribute to the reduced percent PPI caused by this dose of methylphenidate, as no prepulse stimulus reduced startle amplitude at this dose. Additionally, in light of the fact that the high dose of methylphenidate did not uniformly increase startle amplitude in BN rats, the data from this group was split in half so that PPI in the rats with the lowest startle amplitude ($n=6$) could be compared to data from the rats with the highest startle amplitude ($n=5$), and data from both of these sub-groups were compared to data from the saline-treated group. Both PPI and startle data were subjected to ANOVA followed by a least significant difference post-hoc test. Fig. 6A shows that startle amplitude in the methylphenidate-treated rats from the low half of the distribution was equivalent to that in the saline-treated rats. There was a significant effect of group, $F(2,18)=14.0$, $p<0.001$, which was attributable to the significant difference between the methylphenidate-treated rats in that showed high startle amplitude and each of the other two groups. Fig. 6B shows that there was also a significant group effect on percent PPI, $F(2,18)=7.5$, $p=0.004$. For these data, there was a significant difference between the saline-treated group and the low startle methylphenidate group ($p=0.029$), as well as between the saline-treated group and the high startle methylphenidate group ($p=0.002$). There was no significant difference between the two methylphenidate sub-groups, although this may have been due to the large variance in the high startle sub-group.

4. Discussion

The results of the present experiments are in agreement with our previous results, and those of others, showing that relatively low-dose CRF (i.c.v.), disrupts PPI independently of CRF-induced changes in baseline startle amplitude (Conti, 2005; Risbrough et al., 2004). Although the effects of CRF on baseline startle and PPI may be mediated via different brain regions, when CRF-treated animals were matched for startle amplitude with saline-treated animals, CRF diminished PPI. In addition, the present results reveal that repeated i.c.v. infusion of CRF does not cause a rapid or short-term tolerance to this effect of CRF. However, rapid, short-term tolerance to either a single or repeated i.c.v. infusion of CRF develops to its effects on locus coeruleus discharge rate (Conti and Foote, 1995). The finding that tolerance does not develop to the effect of CRF on PPI adds credence to the use of CRF in models of the neurobiology and pharmacology of sensorimotor gating disorders that occur in chronic disorders. The finding also suggests that the effect of CRF on PPI may not involve CRF-induced activation of the locus coeruleus. The lack of tolerance observed with PPI is similar to the lack of tolerance to the locomotor-stimulating or anxiogenic effects of CRF (Buwalda et al., 1998; Song et al., 1995). It remains possible that tolerance would have developed with a longer treatment regimen, as tolerance to the effect of a DA D2 receptor agonists does develop following a 28-day treatment regimen (Culm et al., 2004).

The disruptive effect of apomorphine, and other DA receptor agonists, on PPI has been well-studied (see Geyer et al., 2001 for review). CRF has been shown to enhance both DA utilization and extracellular concentrations of DA (Kalivas et al., 1987; Lavicky and Dunn, 1993; Matsuzaki et al., 1989). Additionally, repeated central infusion of CRF has been shown to enhance or reduce subsequent amphetamine-induced stereotypy, depending on the dose of CRF (Cole and Koob, 1989; Izzo et al., 2005). Thus, it was possible that repeated CRF might alter the effect of apomorphine on PPI. This was not the case in the present study. The effect of apomorphine on PPI was equivalent in rats that had been repeatedly treated with saline or CRF. While the effects of apomorphine on PPI have been studied by a number of investigators, this is the first experiment in which such effects were examined in BN rats. Sensitivity to the PPI-disrupting effects of apomorphine are rat strain-dependent (Rigdon, 1990; Swerdlow et al., 2004, 2005). Here, we show that BN rats are very sensitive to the PPI-disrupting effects of relatively low doses of apomorphine. Since PPI is also disrupted by CRF in BN rats at lower doses than in other strains (Conti, 2005), the BN strain may serve as a useful model to study the pharmacology of PPI.

Sensitization to the effects of apomorphine on subsequent apomorphine-induced disruption of PPI has been reported to occur under some, but not all conditions (Druhan et al., 1998; Martin-Iverson, 1999). Although there was also no apparent sensitization to the effect of CRF on either the CRF- or the apomorphine-induced reduction in PPI, it is possible that there was a floor effect, such that greater disruption of PPI could not be seen under the conditions employed. In future experiments designed to examine potential sensitization, the test day dose of CRF should be a dose that does not acutely disrupt PPI.

CRF has been shown to increase baseline startle amplitude (Liang et al., 1992; Meloni et al., 2006; Swerdlow et al., 1989), although in the reports by Liang et al. and Meloni et al., this effect occurred at a time post-CRF infusion that was later than the time at which we assessed the effects of CRF. Nevertheless, an exogenous CRF-induced decrease in startle amplitude, such as that seen in the present study, has not been reported. This effect of CRF was not seen in rats that had been repeatedly treated with CRF, perhaps because tolerance develops to this effect of CRF. Alternatively, the lack of effect of CRF in this group may have been due to the fact that baseline startle amplitude was somewhat lower in rats that had been repeatedly treated with CRF, than in those repeatedly treated with saline. Interestingly, CRF over-expressing mice, which show diminished PPI compared to WT mice, also show lower baseline startle than WT mice (Dirks et al., 2002). In our previous reported work, CRF had no effect on baseline startle amplitude in BN rats (Conti, 2005). The reason for the CRF-induced decrease in startle amplitude in the present experiments is not clear. Both the CRF_1 and the CRF_2 receptor mediate the startling-enhancing effect of CRF in mice (Risbrough et al., 2003). If the same is true of rats, then it is unlikely that differences in the distribution and/or density two CRF receptors between BN and other rat strains is the reason for behavioral differences among the strains. The effect of CRF on baseline startle was not specific, as apomorphine also decreased startle amplitude in the present study. Others have found that apomorphine has either no effect or enhances baseline startle amplitude (Feifel et al., 1999; Rigdon, 1990).

Although the reason for the CRF-induced decrease in startle amplitude is not clear at this time, it was important to examine

whether this decrease contributed to the decrease in percent PPI. To study this, we examined the effect of CRF on startle and PPI using two startle stimulus intensities in the same testing session. We found that, with the 105 dB startling stimulus, startle amplitude in saline-treated rats was equivalent to that seen in CRF-treated rats following the 120 dB stimulus. Although there was equivalent startle amplitude in these two groups, the CRF-treated group showed significantly less PPI than the saline-treated group. We also found that, while startle amplitude was significantly greater in CRF-treated rats following the 120 dB stimulus than it was following the 105 dB startling stimulus, the prepulse stimulus had no effect on startle amplitude in CRF-treated rats in the 120 dB startle stimulus condition (data not shown). Together, these results reveal that the effect of CRF on PPI is independent of its effects on startle amplitude, confirming the results of Risbrough et al. (2004).

Methylphenidate decreased PPI in both WKY and BN rats at a dose that is comparable to a dose of amphetamine that disrupts PPI, but not at a lower dose. This doses of amphetamine (3.0 mg/kg) had no effect on startle amplitude in one recent study (Swerdlow et al., 2003a), but increased startle amplitude in another (Swerdlow et al., 2003b). However, the 20.0 mg/kg dose of methylphenidate, which decreased PPI, did increase startle amplitude above that found in rats treated with 1.0 mg/kg methylphenidate, which did not affect PPI. Davis (1988) convincingly argues that, when a drug increases baseline startle, a decrease in percent PPI can be an artifact of the increased baseline rather than a real effect of the drug. Although the effective dose of methylphenidate increased baseline startle amplitude in the present study, the effect on percent PPI was not solely due to this increase. This can be seen in Fig. 5, which shows that at the 20.0 mg/kg dose, prepulse stimuli failed to inhibit startle amplitude. Additionally, when startle amplitude in a sub-group of methylphenidate-treated rats was matched for amplitude in the saline-treated rats, percent PPI was still significantly reduced. Nevertheless, it should be noted that the reduction in percent PPI was greater yet in the sub-group of methylphenidate-treated rats in which startle was significantly increased. Thus, following some manipulations, reduced PPI may have a startle-independent component, as well as a startle-dependent component. Since methylphenidate increased startle amplitude in BN rats, it can be concluded that the strain is sensitive to the PPI-disruptive effects of a number of compounds irrespective and independent of the effect that different drugs have on baseline startle. In the present study, methylphenidate also disrupted PPI in WKY rats. This stands in contrast to the results of a study by Drolet et al. (2002) who found that, while methylphenidate decreased PPI in Wistar rats, it was without effect in WKY rats. However, the highest dose of methylphenidate used in the study by Drolet et al. was 5.0 mg/kg, and the effective dose in the present study was 20.0 mg/kg. While doses of methylphenidate, at or below the lower dose used in the present study have been shown to improve sustained attention, and increase extracellular concentration of both DA and norepinephrine in the pre-frontal cortex of rats (Berridge et al., in press), the 1.0 mg/kg dose of methylphenidate had no effect on either PPI or startle amplitude in the present study. This suggests that some doses of psycho-stimulants may not decrease PPI, even if these doses do increase extracellular concentrations of the catecholamines and improve cognitive function.

In summary, the present result show that CRF (i.c.v.) reduces percent PPI even after repeated infusion, suggesting that there is a lack of tolerance to CRF on this measure of sensorimotor gating. Additionally, repeated infusion of CRF failed to diminish the apomorphine-induced reduction in PPI. In the BN rats used in the present studies, CRF decreased, rather enhanced baseline startle amplitude. However, by controlling for startle amplitude, by manipulating the intensity of the startling stimulus, we were able to show that CRF reduced percent PPI even when baseline startle in CRF-treated rats is equivalent to baseline startle in saline-treated rats. This result is consistent with the findings of Risbrough et al. (2004), and further suggests that CRF affects PPI and baseline startle independently. Although both CRF and apomorphine reduced baseline startle in BN rats in the present experiments, methylphenidate did not, and the 20.0 mg/kg dose resulted in baseline startle amplitude that was significantly greater than that seen at the 1.0 mg/kg dose. Nevertheless, percent PPI was only significantly reduced by the 20.0 mg/kg dose. Thus, PPI can be reduced in BN rats by treatments which do not reduce startle amplitude. Further, at the 20.0 mg/kg dose of methylphenidate, prepulse stimuli did not diminish startle amplitude, suggesting that PPI can be affected in BN rats even when baseline startle is enhanced.

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