Restraint stress-induced reduction in prepulse inhibition in Brown Norway rats: Role of the CRF$_2$ receptor

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A B S T R A C T

Stress plays a role in many psychiatric disorders that are characterized by deficits in prepulse inhibition (PPI), a form of sensorimotor gating. Corticotropin-releasing factor (CRF) is one of the most important neurotransmitters involved in behavioral components of the stress response. Central infusion of CRF reduces PPI in both rats and mice. In mice, it has been shown that CRF$_1$ receptor activation mediates the effect of exogenous CRF on PPI. However, the roles of the two CRF receptors in a stress-induced reduction in PPI are not known. We sought to determine whether CRF$_1$ and/or CRF$_2$ receptor blockade attenuates a stress-induced reduction of PPI in rats. In separate experiments, we assessed PPI in Brown Norway rats after exposure to 5 days of 2-h restraint, and after pretreatment with the CRF$_1$ receptor antagonist, CP-154,526 (20.0 mg/kg), or the CRF$_2$ receptor antagonist, antisauvagine-30 (10.0 μg). Repeated, but not acute, restraint decreased PPI and attenuated the increase in PPI caused by repeated PPI testing. Blockade of the CRF$_1$ receptor did not attenuate the effect of repeated restraint on PPI or grooming behavior. While CRF$_2$ receptor blockade did attenuate the effect of repeated restraint on PPI, repeated ICV infusion of the selective CRF$_2$ receptor agonist urocortin III, did not affect PPI. These findings demonstrate the effect of stress on sensorimotor gating and suggest that the CRF$_2$ receptor mediates this effect in rats.

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1. Introduction

The acoustic startle response (ASR) is a reflexive response to an unexpected and intense auditory stimulus and is characterized by contraction of the facial and skeletal muscles (Koch, 1999). Despite the fact that the ASR is a reflex, it can be modulated. For example, startle amplitude is reduced if a non-startling acoustic stimulus is presented shortly prior to the startling stimulus (Hoffman and Ison, 1980; Hoffman and Searle, 1968). This form of startle plasticity, referred to as prepulse inhibition (PPI), is a measure of sensorimotor gating (Braff and Geyer, 1990). Patients with a variety of psychiatric disorders, including schizophrenia (Braff et al., 1978, 1992), obsessive-compulsive disorder (Swerdlow et al., 1993), and post-traumatic stress disorder (Grillon et al., 1996) show less PPI than control subjects. Exposure to stressful events can trigger the onset or exacerbate symptoms of each of these disorders (Dinn et al., 1999; Keane et al., 2006; Walker and Diforio, 1997). Since PPI can be assessed in both humans and rodents under nearly identical parameters, it is a useful tool for investigating deficits in human information processing using animal models.

Corticotropin-releasing factor (CRF) is one of the most important hormones and neurotransmitters involved in endocrine, autonomic, and behavioral components of the stress response (Bale and Vale, 2004; Gray, 1993). A 41-residue peptide, CRF 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 peptide acts at two 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 PPI, including the basolateral amygdala, hippocampus, and frontal cortex (Swerdlow et al., 2001; Van Pett et al., 2000). Intracerebroventricular (ICV) infusion of CRF reduces PPI in both rats (Conti, 2005; Conti et al., 2002) and mice (Risbrough et al., 2004). Additionally, transgenic mice over-expressing CRF show reduced PPI compared to wild-type controls (Dirks et al., 2002).

The first goal of these studies was to investigate the effects of restraint stress on PPI in adult rats. It has previously been shown that PPI is reduced in adult rats, which had undergone early-life stress such as maternal deprivation and social isolation (Weiss and Feldon, 2001). However, studies on the effect of restraint stress in adulthood on PPI have produced inconsistent results (Acri, 1994; Bijlsma et al., 2009; Faraday, 2002; Faraday et al., 1999). We have shown that repeated restraint stress attenuates the increase in PPI caused by repeated PPI testing (Sutherland et al., 2010). Here, we

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examined whether beginning the application of repeated restraint after PPI levels were increased by repeated testing would decrease PPI. Risbrough et al. (2004) have demonstrated that the CRF_1 receptor mediates the reduction in PPI caused by exogenously administered CRF, while the CRF_2 receptor mediates an opposing effect of CRF. However, it is not known whether the two CRF receptors similarly mediate stress-induced changes in PPI or whether the two CRF receptors act similarly in rats and mice. Thus, the second goal of these studies was to determine which CRF receptor mediates the effect of stress on PPI in rats.

In the present studies, we examined whether 2-h restraint stress, administered once a day for 5 consecutive days, decreases PPI. Since PPI increases with repeated testing, we next examined whether restraint stress decreases PPI following repeated testing. We then examined whether and how the two CRF receptors mediate the effect of restraint stress on PPI. Rats were pretreated with either a selective CRF_1 or CRF_2 receptor antagonist (in two separate experiments) prior to restraint exposure on each of 5 consecutive days. Finally, we examined the effect of repeated infusion (ICV) of the selective CRF_2 receptor agonist, urocortin III (Ucn III), on PPI.

We began this examination of the effects of restraint stress on PPI and the potential roles of the two CRF receptors on any such effect using Brown Norway (BN) rats. This rat strain was chosen for several reasons. First, we have extensively characterized the effects of exogenously administered CRF on PPI and startle amplitude in BN rats (Conti, 2005; Conti et al., 2002, 2005, 2006, 2009; Sutherland et al., 2008). Thus, comparisons can be made between the findings of this study and our previously published studies. Second, BN rats are more sensitive to the effects of ICV infusion of CRF (Conti et al., 2002; Sutherland et al., 2008) and to the effects of stress (Sutherland et al., 2010) on PPI than Wistar-Kyoto (WKY) rats, another commonly used strain in our laboratory. This sensitivity to CRF and stress suggests that the BN rat could be developed as a model of stress-induced exacerbation of psychiatric disorders in which there are deficient sensorimotor gating phenotypes.

2. Materials and methods

2.1. Animals

A total of 163 male Brown Norway (BN) rats (Harlan Sprague-Dawley, Prattville, AL, USA) was 10 weeks old upon arrival and was 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 or restraint exposure, and single-housed thereafter. All procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Experimental design

In experiment 1, rats were restrained for 2 h, once a day for 5 consecutive days, by being placed into acrylic cylindrical restrainers designed for rodents weighing 250–500 g (6.4 cm diameter, with adjustable length; Biomedical Research Instruments, Silver Springs, MD, USA). Control rats were handled briefly but not restrained. Rats were tested for PPI and startle amplitude 30 min after restraint termination on days 1, 3, and 5.

In experiment 2, PPI and startle amplitude were assessed once a day for 5 consecutive days, with no restraint being imposed. After day 5 of PPI testing, the rats were divided into two counter-balanced groups based on average percent PPI. Two days later, half of the rats underwent 2-h restraint/day for 10 consecutive days, and the other half served as controls that were not restrained. Rats were again tested for PPI 30 min after restraint termination on days 1, 3, 6, and 10.

In experiment 3, rats were administered a subcutaneous (SC) injection of the non-peptide selective CRF_1 receptor antagonist, CP-154,526 (20.0 mg/kg) (Schulz et al., 1996; Seymour et al., 2003), or vehicle. Forty-five minutes after injection, rats were restrained for 2 h or were handled briefly but not restrained. Rats were subjected to injection and restraint (or brief handling) once a day for 5 consecutive days, and were tested for PPI and startle amplitude 30 min after restraint termination on days 1, 3, and 5. Fifteen minutes prior to PPI testing, grooming behavior was observed for 15 min as a secondary behavioral measure of stress (Spruijt et al., 1992).

Experiment 4 was conducted to examine the effect of CP-154,526 on a CRF-induced decrease in PPI. Rats were administered an SC injection of CP-154,526 (20.0 mg/kg) or vehicle. Two hours and 45 min after injection, rats received an ICV infusion of 0.3 µg CRF (in 6.0 µl saline) or 6.0 µl saline. Rats were tested for PPI and startle amplitude 30 min after ICV infusion. Grooming behavior was observed for 15 min immediately before PPI testing as a secondary behavioral measure of the effect of CRF (Dunn et al., 1987). Injection of CP-154,526 2 h and 45 min before CRF infusion ensured that the time between SC injection and PPI testing was the same in all experiments in which CP-154,526 was used.

Experiment 5 was conducted to replicate previous findings that a selective CRF_1 receptor antagonist blocks the CRF-induced increase in anxiety-like behavior in the elevated plus maze (Zorrilla et al., 2002). Rats were administered an SC injection of CP-154,526 (20.0 mg/kg) or vehicle. Two hours and 45 min after injection, rats received an ICV infusion of 1.0 µg CRF (in 5.0 µl saline) or 5.0 µl saline. Ten minutes after ICV infusion, rats were observed in the elevated plus maze for 5 min.

In experiment 6, rats received an ICV infusion of the selective CRF_2 receptor antagonist, antisauvagine-30 (ASV-30; 10.0 µg in 5.0 µl saline) (Higelin et al., 2001), or 5.0 µl saline. Ten minutes after ICV infusion, rats were restrained for 2 h or were handled briefly but not restrained. Rats were subjected to infusion and restraint (or brief handling) once a day for 5 consecutive days, and were tested for PPI and startle amplitude 30 min after restraint termination on days 1, 3, and 5. Fifteen minutes prior to PPI testing, grooming behavior was observed for 15 min.

Experiment 7 was conducted to examine the effect of repeated administration of a selective CRF_2 receptor agonist, Ucn III, on PPI. Rats received an ICV infusion of 20.0 µg Ucn III (in 5.0 µl saline) or 5.0 µl saline once a day for 5 consecutive days. Rats were tested for PPI and startle amplitude on days 1, 3 and 5 beginning 2.5 h after ICV infusion.

2.3. Drugs and peptides

CP-154,526 (N-butyl-N-[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]-N-ethylamine) was generously donated by Pfizer, Inc (Groton, CT, USA). In a heated water bath (65 °C), a stock solution was prepared by dissolving CP-154,526 in DMSO (Sigma−Aldrich, St. Louis, MO, USA) and Cremophor (Sigma−Aldrich) in the ratio 90:5:5 (100 mg/ml), and was stored at room temperature. On each testing day, stock solution was placed into a microcentrifuge tube and distilled water (heated to 60 °C) was added to reach a concentration of 10 mg/ml. Rats were lightly anesthetized with isoflurane-in-oxygen (5%) for 90 s prior to SC injection. Rat/human CRF, kindly provided by Dr. Jean Rivier (The Salk Institute, La Jolla, CA, USA), ASV-30 (Sigma−Aldrich) and mouse Ucn III (Bachem, Torrance, CA, USA) were dissolved in 0.9% saline. Aliquots of each peptide were frozen at −80 °C until needed.

2.4. 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. To minimize pain, rats received an SC injection of buprenorphine (0.05 mg/kg) while anesthetized. Rats recovered 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, ASV-30, or Ucn III over 1 min. 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.

2.5. Startle chambers 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.

Following a 5-min acclimation period, stimuli were delivered over a 70 dB white noise background. The first and last six trials of the session consisted of the startle stimulus alone (120 dB, 40 ms). Remaining trials occurred in a pseudorandom order and consisted of 12 startle alone trials (used to calculate % PPI and average startle amplitude), 12 prepulse + startle trials at each of 4 prepulse intensities (76, 82, 85, 88 dB), and 8 no stimulus trials. Prepulse stimuli (20 ms) preceded startle stimuli by

100 ms. The inter-trial interval averaged 20 s. Testing was performed between 10 a.m. and 4 p.m.

2.6. Elevated plus maze

An elevated plus maze, made of white plastic (San Diego Instruments), was elevated to a height of 49.5 cm, with two open (50.2 × 10.2 cm) and two enclosed arms (50.2 × 10.2 cm, walls were 30.5 cm high). A 70-W desk lamp was placed 0.9 m from the maze, with the bulb angled away from the maze, to create dim lighting conditions in the small room. During observation, the experimenter always sat in the same place next to the maze. Only the rat being tested was brought into the maze room and each rat was naïve to the apparatus. Rats were placed in the center of the maze facing the same enclosed arm. During the 5-min observation period, the amount of time spent in the open arms, as well as the total number of open and closed arm entries, were recorded. An entry required that all four limbs of the rat be in that arm.

2.7. Data and statistical analyses

Percent PPI was calculated for each rat at each prepulse intensity using the following equation: % PPI = 100 − (100 × [$prepulse/startle$]). $Prepulse$ was the average startle amplitude on trials in which a prepulse stimulus preceded the startle stimulus. $Startle$ was the average amplitude on trials in which the startle stimulus was presented alone, excluding the first and last 6 trials. In order to examine whether selective CRF receptor antagonists or stress altered habituation of the startle response in experiments 3 and 6, percent habituation was calculated as: 100 × (average of first 6 startle trials − average of last 6 startle trials/average of last 6 startle trials).

Data were analyzed using one-, two-, three-, or four-way analysis of variance (ANOVA), as discussed in detail in the Results section. Tukey's post-hoc tests were performed if significant main effects were found. Independent t-tests with Bonferroni correction were used where appropriate. In all experiments assessing PPI, the average of all prepulse stimulus intensities (76, 82, 85, and 88 dB) is shown in the figures as 'Percent Prepulse Inhibition' to allow for easier visualization of the main statistical findings. Interactions with prepulse intensity are reported in the text and occur because experimental effects, such as restraint, are greater at the 76, 82, and 85 dB prepulse intensities than at the 88 dB intensity. Additionally, main effects of prepulse intensity, which occur because percent PPI increases with increasing prepulse intensity, are not reported since they are statistically significant in all analyses conducted.

3. Results

3.1. Experiment 1: effect of five consecutive days of restraint stress on PPI and startle amplitude

Fig. 1 shows that prepulse inhibition increased over days of testing and this increase was attenuated by repeated restraint. Acute restraint did not affect PPI. A three-way ANOVA was used to analyze PPI data from all 3 testing days, with restraint as a between-subjects factor and day and prepulse intensity as within-subjects factors (Fig. 1). Significant main effects of restraint [$F(1,18) = 10.71$; $p = 0.005$] and day [$F(2,36) = 18.86$; $p < 0.001$] on PPI were detected. There were trends toward interactions between day and restraint ($p = 0.053$), day and prepulse intensity ($p = 0.088$), and among day, prepulse intensity, and restraint ($p = 0.067$). In order to determine on which days restraint affected PPI, data from each day were examined separately using two-way ANOVAs.

A two-way ANOVA showed that restraint did not alter PPI on day 1 (Fig. 1). On day 3, restraint significantly attenuated the increase in PPI caused by repeated testing [$F(1,18) = 21.13$; $p < 0.001$] (Fig. 1). A significant prepulse intensity × restraint interaction [$F(3,54) = 4.11$; $p < 0.02$] indicated that the effect of restraint on PPI was more robust at lower prepulse intensities (data not shown). On day 5, there was a trend for restraint to attenuate the increase in PPI caused by repeated testing ($p = 0.094$) (Fig. 1). Analysis of startle amplitude data (not shown) using a two-way ANOVA showed a significant effect of day [$F(2,36) = 4.24$; $p < 0.05$], indicating that startle amplitude diminished as the days of testing progressed due to habituation. Restraint stress did not alter startle amplitude on any day.
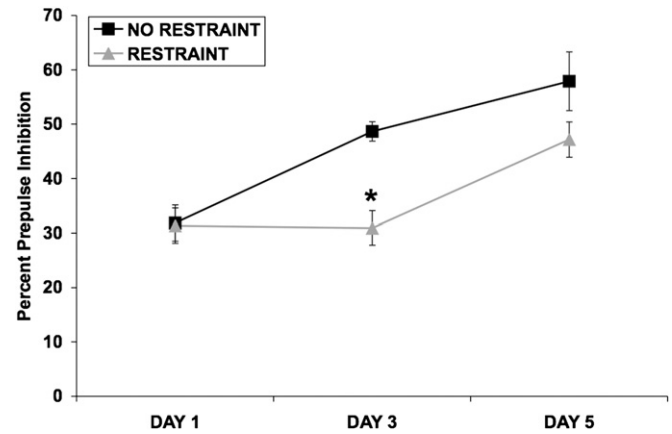

Fig. 1. Effect of 5 consecutive days of restraint stress on PPI. Values shown are means ± SEMs. For all groups, n = 9–11. The average of all prepulse stimulus intensities (76, 82, 85, and 88 dB) is shown as Percent Prepulse Inhibition. Rats were restrained for 2 h/day for 5 consecutive days, or were handled briefly and returned to the home cage. PPI was assessed 30 min after restraint termination on days 1, 3, and 5. On day 1, restraint did not alter PPI. On day 3, restraint significantly attenuated the increase in PPI caused by repeated testing (*$p < 0.001$ vs. No Restraint on day 3). On day 5, there was a trend for restraint to attenuate the increase in PPI caused by repeated testing.

3.2. Experiment 2: effect of restraint stress on PPI following repeated PPI testing

In this experiment, the restraint stress sessions began after rats had been repeatedly tested for PPI on each of 5 consecutive days. This was done so that restraint would only begin after the repeated testing-induced increase in PPI had been achieved. Under these conditions, repeated restraint decreased PPI (Fig. 2). Thus, repeated restraint does not merely attenuate a testing-induced increase in PPI. A two-way ANOVA, with restraint as the between-subjects factor and day as the within-subjects factor, was used to analyze PPI
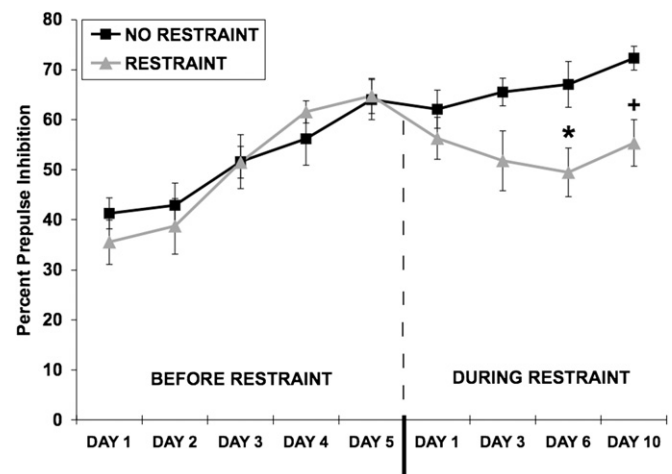

Fig. 2. Effect of restraint stress on PPI following repeated PPI testing. Values shown are means ± SEMs. For all groups, n = 8. The average of all prepulse stimulus intensities (76, 82, 85, and 88 dB) is shown as Percent Prepulse Inhibition. PPI was assessed once a day, for 5 consecutive days, in the absence of restraint (left side of dash). After day 5 of testing, the rats were divided into two counter-balanced groups based on average percent PPI. After a 2-day rest period, one group of rats was exposed to 2-h restraint/day for 10 consecutive days (right side of dash). Rats in the control group were handled briefly, but not restrained. Rats were tested for PPI 30 min after restraint termination on days 1, 3, 6, and 10. Once restraint exposure began, PPI was decreased in restrained rats compared to non-restrained rats on day 6 (*$p = 0.02$) and on day 10 (+$p < 0.01$).

data obtained from the first 5 days of testing, prior to restraint (Fig. 2, left side of dash). A significant main effect of day [$F(4,56) = 31.0$; $p < 0.001$] showed that PPI increased over the 5 days of testing. PPI was not different between the two groups prior to the start of restraint (Fig. 2, left side of dash). A two-way ANOVA conducted on startle amplitude data revealed a significant effect of day [$F(4,56) = 19.29$; $p < 0.001$], since startle decreased over the days of testing due to habituation (data not shown). In stress-naïve rats, average percent PPI after day 5 of testing (64.01 ± 4.02; Fig. 2, left side of dash) was comparable to that seen in experiment 1 after day 5 of testing (57.88 ± 5.40; Fig. 1).

After a 2-day rest period, rats were exposed to 2-h restraint stress/day for 10 consecutive days (Restraint) or were handled briefly but not restrained (No Restraint) (Fig. 2, right side of dash). A two-way ANOVA conducted on PPI data from days 1, 3, 6, and 10 of restraint revealed a significant main effect of restraint [$F(1,14) = 9.56$; $p < 0.01$]. A three-way ANOVA conducted on PPI data from day 5 pre-restraint and days 1, 3, 6, and 10 of restraint revealed a significant main effect of restraint [$F(1,14) = 6.60$, $p < 0.05$] and a significant restraint × day interaction [$F(4,42) = 2.77$, $p < 0.05$]. The levels of PPI did not continue to increase significantly in the non-restrained group over days 1, 3 and 6, while the experimental group was undergoing restraint ($p > 0.05$). However, if day 10 of restraint was included in the analysis, the non-restrained group showed an increase in PPI from day 5 of pre-restraint, [$F(4,28) = 3.96$, $p < 0.02$]. Separate ANOVAs were used to determine on which days during restraint the restrained and non-restrained groups differed from each other and revealed that there was a marginal difference between the groups on day 3 ($p = 0.056$), a significant difference on day 6 [$F(1,14) = 6.94$, $p = 0.02$], and a significant difference on day 10 [$F(1,14) = 10.56$, $p < 0.01$], although the increase in PPI in the non-restrained group may have contributed to the difference on this day of testing. These results suggest that repeated restraint decreased PPI in addition to blunting the increase in PPI caused by repeated testing (Experiment 1). A separate two-way ANOVA showed that restraint did not alter startle amplitude on any day (data not shown).

3.3. Experiment 3: effect of restraint stress and selective CRF₁ receptor blockade on PPI, startle amplitude, and grooming behavior

Again in this experiment, there was no effect of restraint on PPI on day 1, but significant effects on days 3 and 5 (Fig. 3). The CRF₁ receptor antagonist, CP-154,526, did not attenuate the effect of stress on any test day. A four-way ANOVA was used to analyze PPI data from all 3 testing days (Fig. 3), with SC injection and restraint as between-subjects factors, and day and prepulse intensity as within-subjects factors. Significant main effects of restraint [$F(1,36) = 18.27$; $p < 0.001$] and day [$F(2,72) = 14.61$; $p < 0.001$] on PPI were detected. There were significant interactions between prepulse intensity and restraint [$F(3,108) = 3.76$; $p < 0.02$] and between day and prepulse intensity [$F(6,216) = 3.47$; $p < 0.005$]. To determine on which days restraint affected PPI, data from each day were examined separately using three-way ANOVAs.

On day 1 (Fig. 3a), neither pretreatment with CP-154,526 nor restraint altered PPI, as no significant main effects or interactions were found. Restraint attenuated the increase in PPI caused by repeated testing on day 3 [$F(1,36) = 16.62$; $p < 0.001$] (Fig. 3b) and on day 5 [$F(1,36) = 10.67$; $p = 0.002$] (Fig. 3c). On day 5, a significant prepulse × restraint interaction [$F(3,108) = 2.82$; $p < 0.05$] was detected. Pretreatment with CP-154,526 did not alter the effect of restraint on PPI observed on days 3 and 5 of restraint. Analysis of startle amplitude data using a three-way ANOVA revealed a significant effect of day [$F(2,72) = 18.64$; $p < 0.001$], indicating that
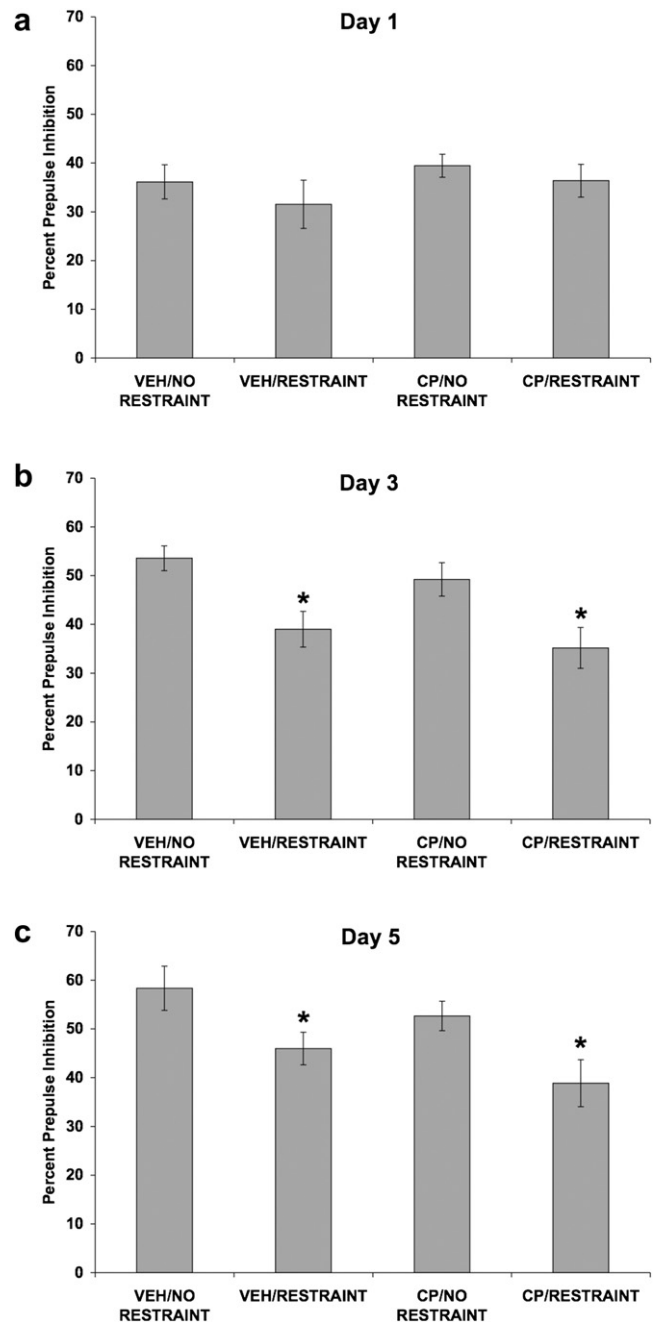

Fig. 3. Effect of restraint stress and selective CRF₁ receptor blockade on PPI. Values shown are mean ± SEMs. For all groups, $n = 10$. The average of all prepulse stimulus intensities (76, 82, 85, and 88 dB) is shown as Percent Prepulse Inhibition. X-axis shows Pretreatment (SC injection)/Treatment (absence or presence of restraint). Rats were administered an SC injection of CP-154,526 (20.0 mg/kg) or vehicle. Forty-five minutes later, rats were restrained for 2 h or were handled briefly and returned to the home cage. Rats were subjected to injection and restraint (or brief handling) once a day for 5 consecutive days. Rats were tested for PPI 30 min after restraint termination on days 1, 3, and 5. (a) On day 1, neither pretreatment with CP-154,526 nor restraint altered PPI. (b) On day 3, restraint attenuated the decrease in PPI caused by repeated testing (*$p < 0.001$ vs. No Restraint, main effect). CP-154,526 pretreatment did not alter the effect of restraint on PPI. (c) On day 5, restraint attenuated the increase in PPI caused by repeated testing (*$p < 0.01$ vs. No Restraint, main effect). CP-154,526 pretreatment did not alter the effect of restraint on PPI.

startle amplitude decreased over the 3 days of testing due to habituation (Fig. 4). Neither pretreatment with CP-154,526 nor restraint altered startle amplitude. Percent habituation of startle amplitude for all three days of testing is shown in Table 1. There
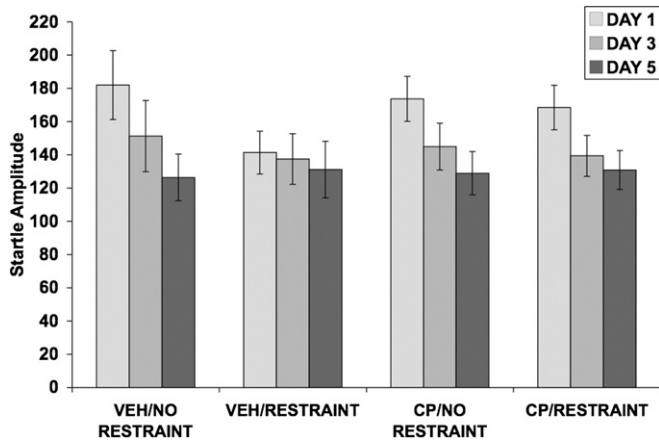
Fig. 4. Effect of restraint stress and selective CRF_1 receptor blockade on baseline startle amplitude. Values shown are means ± SEMs and were calculated from the startle stimulus alone trials that were used to calculate percent PPI. Startle amplitude was assessed 30 min after restraint termination on days 1, 3, and 5. Neither pretreatment with CP-154,526 nor restraint altered startle amplitude.

Fig. 5. Effect of restraint stress and selective CRF_1 receptor blockade on time spent grooming (in seconds). Values shown are means ± SEMs. For all groups, $n = 10$. Grooming was assessed for 15 min, beginning immediately after restraint termination on days 1, 3, and 5. Restrained groups spent more time grooming than control groups ($*p < 0.001$ vs. No Restraint, main effect).

were no significant main effects of SC injection or restraint and no interaction between the two factors.

Data for time spent grooming were analyzed using a three-way ANOVA (Fig. 5). Significant main effects of restraint [$F(1,36) = 28.69; p < 0.001$] and day [$F(2,72) = 8.40; p = 0.001$], as well as significant interactions between SC injection and restraint [$F(1,36) = 4.58; p < 0.05$] and between day and restraint [$F(2,72) = 5.35; p < 0.01$], were found.

3.4. Experiment 4: effect of CRF and selective CRF_1 receptor blockade on PPI, startle amplitude, and grooming behavior

In this experiment, we examined whether the CRF_1 receptor antagonist would attenuate the effect of acute exogenous CRF (ICV) administration on PPI. CRF decreased PPI and this effect was not blocked by CP-154,526 (Fig. 6). A three-way ANOVA, with SC injection and ICV infusion as between-subjects factors and prepulse intensity as a within-subjects factor, was used to analyze PPI data (Fig. 6a). ICV infusion of CRF decreased PPI [$F(1,22) = 5.63; p < 0.05$]. A two-way ANOVA showed that CRF infusion decreased startle amplitude [$F(1,22) = 6.90; p < 0.02$] (Fig. 6b). A two-way ANOVA demonstrated that rats given an ICV infusion of CRF spent more time grooming than the control rats receiving ICV Saline [$F(1,21) = 28.10; p < 0.001$] (data not shown). Pretreatment with
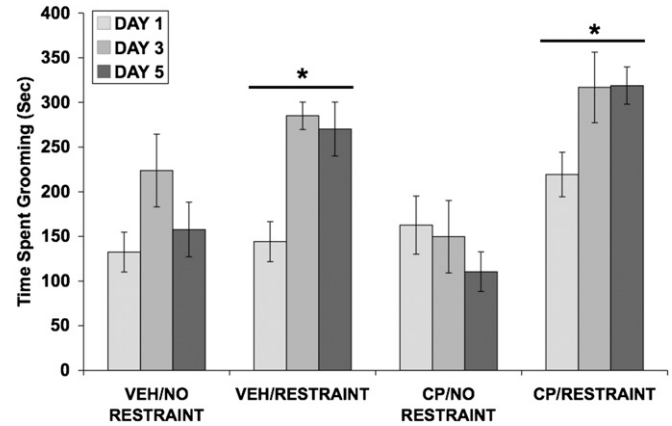
CP-154,526 did not alter the CRF-induced decrease in PPI or startle amplitude, or the CRF-induced increase in grooming behavior.

3.5. Experiment 5: effect of CRF and selective CRF_1 receptor blockade in the elevated plus maze

The effect of CP-154,526 on a CRF-induced increase in anxiety-like behavior in the elevated plus maze was also examined. Alone, CRF significantly reduced the percentage of time that rats spent in the open arms of the maze, and this effect was blocked by the CRF_1 receptor antagonist (Fig. 7). Data for percentage of time spent in the open arms of the elevated plus maze (Fig. 7a) were analyzed using a two-way ANOVA, with SC injection and ICV infusion as between-subjects factors. Significant main effects of SC injection [$F(1,31) = 4.40; p < 0.05$] and ICV infusion [$F(1,31) = 8.90; p < 0.01$] were shown. CRF infusion decreased the percentage of time spent in the open arms of the maze only in vehicle-pretreated rats [$t(15) = 2.95; p = 0.01$], as shown by a separate independent t-test with Bonferroni correction comparing VEH/SALINE to VEH/CRF. Since the CP/SALINE and CP/CRF groups did not differ significantly by a separate independent t-test, CP-154,526 pretreatment attenuated the CRF-induced increase in anxiety-like behavior in the elevated plus maze. A two-way ANOVA showed that neither CP-154,526 injection nor CRF infusion altered the number of closed arm entries in the elevated plus maze (Fig. 7b).

3.6. Experiment 6: effect of restraint stress and selective CRF_2 receptor blockade on PPI, startle amplitude, and grooming behavior

In this experiment we examined whether the selective CRF_2 receptor antagonist, ASV-30, would attenuate the effect of repeated restraint on PPI. Once again, Fig. 8 shows that repeated, but not acute, restraint had a significant effect on PPI and that ASV-30 blocked this effect on day 5, but not on day 3, of restraint. A four-way ANOVA was used to analyze PPI data from all 3 days of testing (Fig. 8), with ICV infusion and restraint as between-subjects factors, and day and prepulse intensity as within-subjects factors. Significant main effects of restraint [$F(1,30) = 7.37; p < 0.02$] and day [$F(2,60) = 27.47; p < 0.001$] on PPI were detected. Additionally, there were significant interactions between day and ICV infusion [$F(2,60) = 3.34; p < 0.05$], between prepulse intensity and restraint [$F(3,90) = 5.90; p = 0.001$], between day and prepulse intensity [$F(6,180) = 4.56; p < 0.001$], and among day, prepulse intensity, and restraint [$F(6,180) = 3.65;$

Table 1
Percent habituation of the startle response (mean ± SEM) from the first block of 6 trials to the last block of 6 trials in Experiments 3 and 6. There were no effects of either CP-154,526 or ASV-30 pretreatment or restraint stress on percent habituation in either experiment.

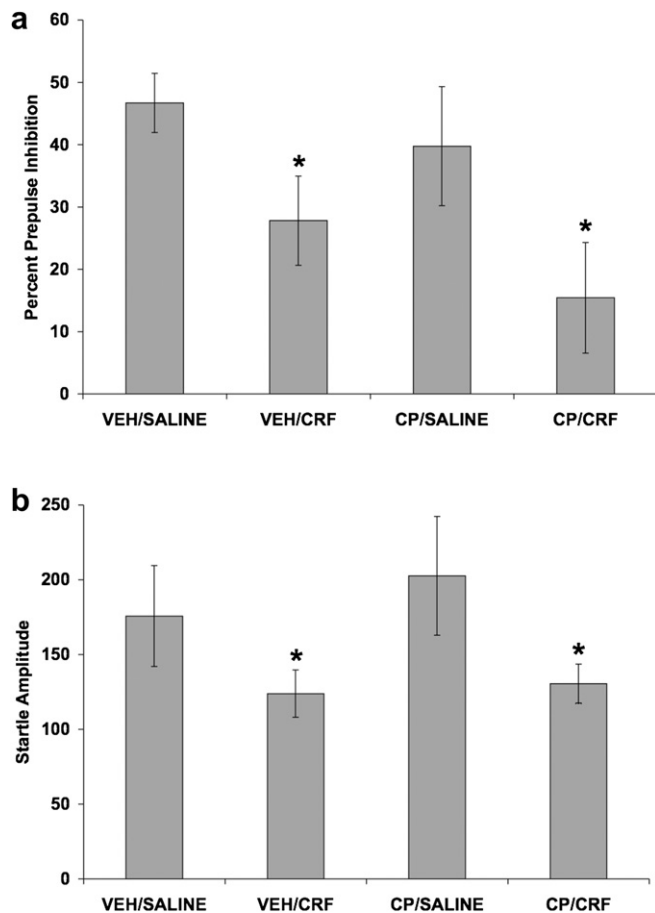| Experiment 3			
Treatment	Day 1	Day 3	Day 5
VEH/NO RESTRAINT	99.05 ± 23.18	81.00 ± 30.12	111.35 ± 38.05
VEH/RESTRAINT	124.10 ± 16.83	111.33 ± 26.17	88.02 ± 20.80
CP/NO RESTRAINT	74.26 ± 17.96	96.04 ± 21.06	159.77 ± 55.55
CP/RESTRAINT	80.15 ± 16.76	134.82 ± 33.89	91.22 ± 32.57
Experiment 6			
Treatment	Day 1	Day 3	Day 5
SAL/NO RESTRAINT	42.63 ± 26.42	96.68 ± 22.93	61.45 ± 28.17
SAL/RESTRAINT	70.10 ± 19.97	55.84 ± 7.57	51.77 ± 17.32
ASV-30/NO RESTRAINT	26.54 ± 15.39	76.31 ± 48.16	41.28 ± 19.73
ASV-30/RESTRAINT	68.02 ± 11.58	24.34 ± 11.54	49.74 ± 23.81

Fig. 6. Effect of CRF and selective CRF_1 receptor blockade on PPI and baseline startle amplitude. Values shown are mean $\pm$ SEMs. For all groups, $n = 4$–9. X-axis shows Pretreatment (SC injection)/Treatment (ICV infusion). Rats received an SC injection of CP-154,526 (20.0 mg/kg) or vehicle. Two hours and 45 min later, rats received an ICV infusion of 0.3 μg CRF (in 6.0 μl saline) or 6.0 μl saline. Rats were tested for PPI and startle amplitude 30 min after ICV infusion. (a) The average of all prepulse stimulus intensities (76, 82, 85, and 88 dB) is shown as Percent Prepulse Inhibition. ICV infusion of CRF decreased PPI (*$p < 0.05$ vs. Saline, main effect). CP-154,526 pretreatment did not alter the CRF-induced decrease in PPI. (b) Startle amplitude on startle stimulus alone trials that were used to calculate percent PPI. ICV infusion of CRF decreased startle amplitude (*$p < 0.05$ vs. Saline, main effect). CP-154,526 pretreatment did not alter the CRF-induced decrease in startle amplitude.
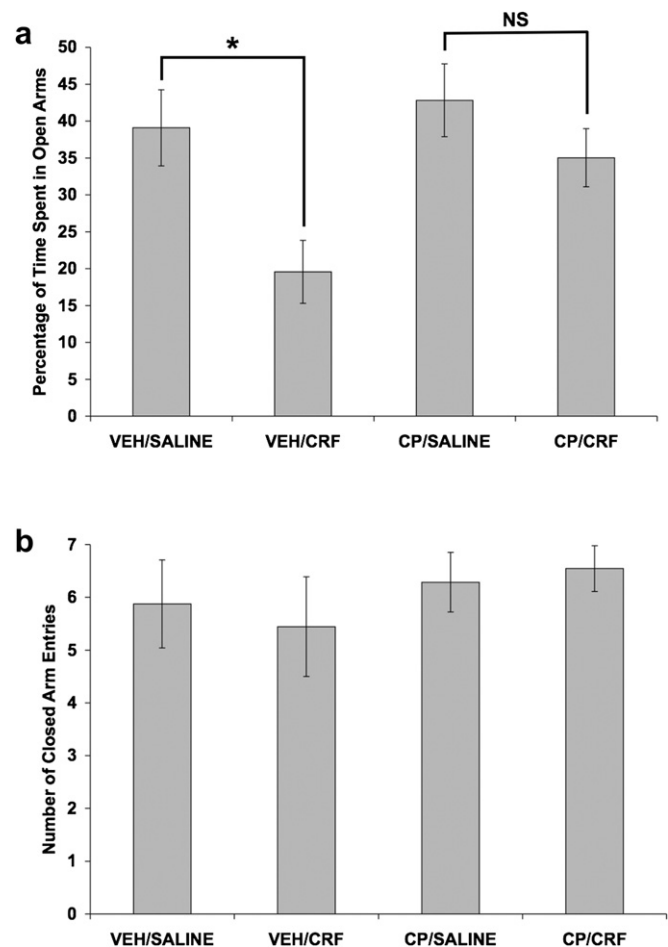
$p = 0.002$]. In order to understand this wide array of interactions, PPI data from each day were examined separately using three-way ANOVAs.

On day 1 (Fig. 8a), there was no effect of ASV-30 infusion or restraint and no interaction between these two factors. On day 3 (Fig. 8b), restraint attenuated the increase in PPI due to repeated testing [$F(1,30) = 6.75$; $p < 0.02$]. A significant prepulse $\times$ restraint interaction [$F(3,90) = 3.92$; $p = 0.011$] indicated that the effect of restraint on PPI was more robust at the lower prepulse intensities. On day 5 (Fig. 8c), there were significant main effects of ICV infusion [$F(1,30) = 4.33$; $p < 0.05$] and restraint [$F(1,30) = 6.25$; $p < 0.02$], and a significant prepulse $\times$ restraint interaction [$F(3,90) = 8.56$; $p < 0.001$]. Separate two-way ANOVAs were conducted to determine whether the effect of restraint was present only in saline-pretreated rats. Indeed, restraint decreased PPI only in rats that received ICV saline, as a significant difference was present between the SAL/NO RESTRAINT and SAL/RESTRAINT groups [$F(1,15) = 7.10$; $p < 0.02$] and was absent between the ASV/NO RESTRAINT and ASV/RESTRAINT groups. Thus, ASV-30 pretreatment blocked the restraint-induced decrease in PPI on day 5 of restraint.

Fig. 7. Effect of CRF and selective CRF_1 receptor blockade on anxiety-like behavior in the elevated plus maze. Values shown are mean $\pm$ SEMs. For all groups, $n = 7$–11. X-axis shows Pretreatment (SC injection)/Treatment (ICV infusion). Rats received an SC injection of CP-154,526 (20.0 mg/kg) or vehicle. Two hours and 45 min later, rats received an ICV infusion of 1.0 μg CRF (in 5.0 μl saline) or 5.0 μl saline. Ten minutes after ICV infusion, rats were observed in the elevated plus maze for 5 min (a) CRF infusion decreased the percentage of time spent in the open arms of the maze only in vehicle-pretreated rats (*$p = 0.010$, independent t-test comparing VEH/SALINE to VEH/CRF). Since CP/SALINE and CP/CRF groups did not differ significantly (NS), CP-154,526 pretreatment blocked the CRF-induced increase in anxiety-like behavior in the elevated plus maze. (b) Neither CP-154,526 injection nor CRF infusion altered the number of closed arm entries.

Analysis of startle amplitude data (Fig. 9) using a three-way ANOVA demonstrated that restraint decreased startle amplitude [$F(1,30) = 10.54$; $p < 0.01$]. A significant effect of day [$F(2,60) = 30.12$; $p < 0.001$] showed that startle amplitude decreased as the days of testing progressed due to habituation. ASV-30 pretreatment did not alter the restraint-induced decrease in startle amplitude. Percent within-session habituation of startle amplitude for all three days of testing is shown in Table 1. There were no significant main effects of ICV infusion or restraint and no interaction between the two factors. There was a significant day $\times$ restraint interaction [$F(2,60) = 3.298$; $p < 0.05$]. Separate two-way ANOVAs conducted on data from days 1, 3, and 5 revealed that this interaction was due to a trend for restraint to increase habituation on day 1 ($p = 0.078$) and a trend for restraint to decrease habituation on day 3 ($p = 0.086$).

Fig. 10 shows the effect of restraint with and without ASV-30 pretreatment on time spent grooming. A three-way ANOVA demonstrated that the restrained groups spent more time grooming than the control groups [$F(1,30) = 62.88$; $p < 0.001$]. A significant effect of day [$F(2,60) = 38.53$; $p < 0.001$] indicated that time
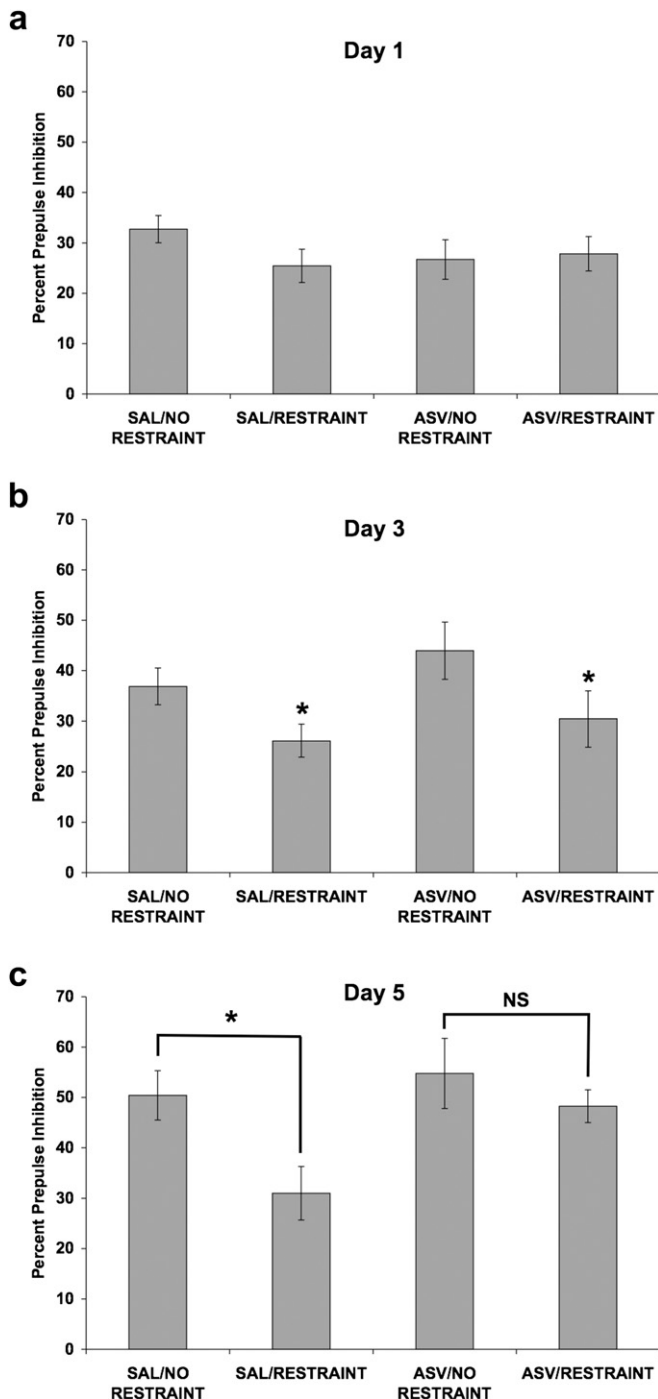
Fig. 9. Effect of restraint stress and selective CRF$_2$ receptor blockade on baseline startle amplitude. Values shown are mean ± SEMs and were calculated from the startle stimulus alone trials that were used to calculate percent PPI. X-axis shows Pretreatment (ICV infusion)/Treatment (absence or presence of restraint). Startle amplitude was assessed 30 min after restraint termination on days 1, 3, and 5. Restraint decreased startle amplitude (*$p < 0.01$ vs. No Restraint, main effect). ASV-30 pretreatment did not alter the restraint-induced decrease in startle amplitude.

spent grooming increased over the three observation periods and a significant day × restraint interaction [$F(2,60) = 17.82; p < 0.001$] showed that restraint increased grooming to a greater extent as the days progressed. ASV-30 pretreatment did not alter the restraint-induced increase in grooming behavior.

3.7. Experiment 7: effect of repeated Ucn III infusion on PPI and startle amplitude

Fig. 11a shows that repeated ICV infusion of Ucn III did not alter PPI on any test day. Ucn III was infused once a day for 5 consecutive days, and PPI was assessed on days 1, 3, and 5. ANOVA with ICV infusion (saline vs. Ucn III) as a between-subjects factor showed no effect of Ucn III ($p > 0.05$), and there were no significant interactions involving the ICV infusion factor. Fig. 11b shows the effect of Ucn III on baseline startle amplitude. Here, there was a significant effect of Ucn III [$F(1,10) = 8.26, p < 0.02$], and a significant effect of day [$F(2,20) = 15.40, p < 0.001$], but no interaction with day.

Fig. 8. Effect of restraint stress and selective CRF$_2$ receptor blockade on PPI. Values shown are mean ± SEMs. For all groups, $n = 8–9$. The average of all prepulse stimulus intensities (76, 82, 85, and 88 dB) is shown as Percent Prepulse Inhibition. X-axis shows Pretreatment (ICV infusion)/Treatment (absence or presence of restraint). Rats received an ICV infusion of 10.0 μg ASV-30 (in 5.0 μl saline) or 5.0 μl saline. Ten minutes later, rats were restrained for 2 h or were handled briefly and returned to the home cage. Rats were subjected to infusion and restraint (or brief handling) once a day for 5 consecutive days. Rats were tested for PPI 30 min after restraint termination on days 1, 3, and 5. (a) On day 1, neither pretreatment with ASV-30 nor restraint altered PPI. (b) On day 3, restraint attenuated the decrease in PPI caused by repeated testing (*$p < 0.05$ vs. No Restraint, main effect). ASV-30 pretreatment did not alter the effect of restraint on PPI on day 3. (c) On day 5, restraint decreased PPI only in rats that received ICV saline, as a significant difference was present between the SAL/NO RESTRAINT and SAL/RESTRAINT groups (*$p = 0.018$) and was absent (NS) between the ASV/NO RESTRAINT and ASV/RESTRAINT groups (separate two-way ANOVAs). Thus, ASV-30 pretreatment blocked the restraint-induced decrease in PPI on day 5 of restraint.
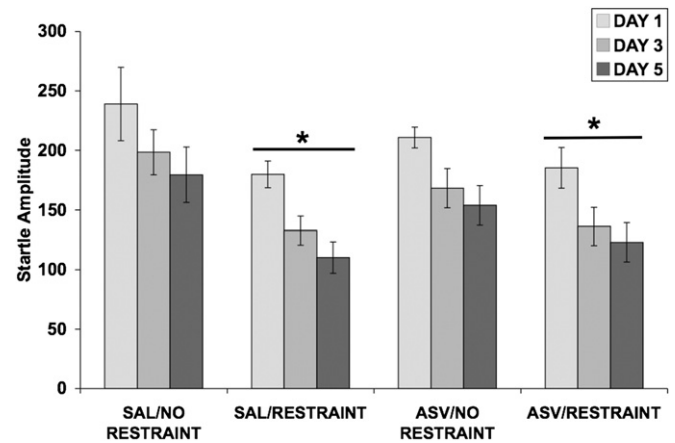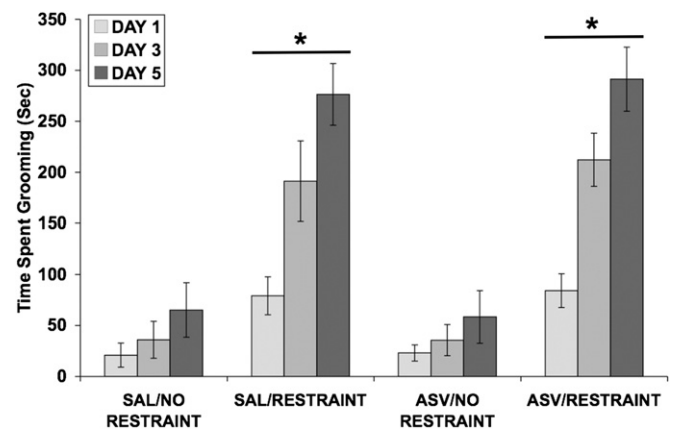

Fig. 10. Effect of restraint stress and selective CRF$_2$ receptor blockade on time spent grooming (in seconds). Values shown are means ± SEMs. For all groups, $n = 8–9$. Grooming was assessed for 15 min, beginning immediately after restraint termination on days 1, 3, and 5. Restrained rats spent more time grooming than control rats (*$p < 0.001$ vs. No Restraint, main effect). Administration of ASV-30 prior to restraint did not alter the restraint-induced increase in grooming behavior.
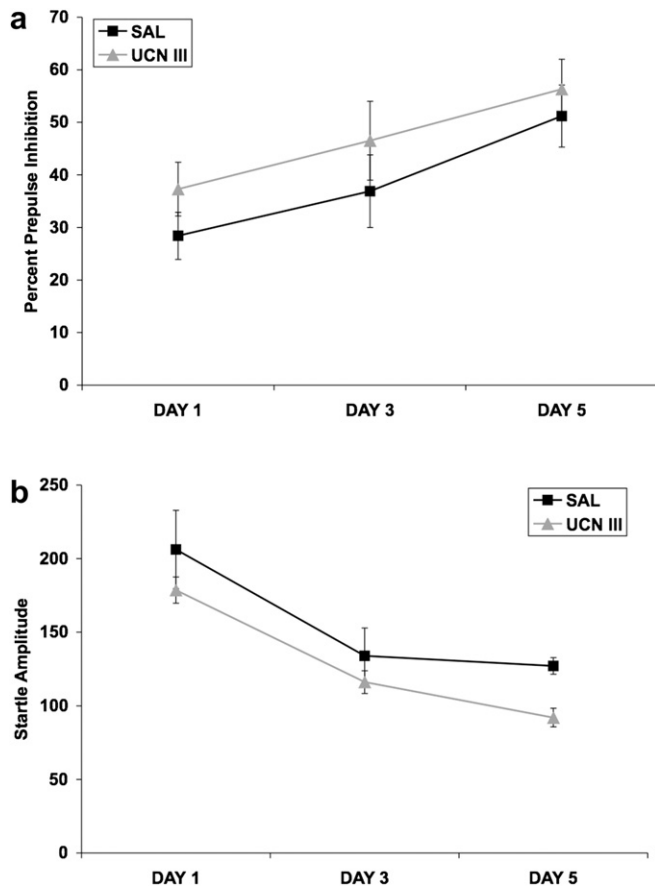
Fig. 11. Effect of repeated ICV infusion of Ucn III on PPI (a) and startle amplitude (b). Values shown are means ± SEMs. For all groups, $n = 6$. The average of all prepulse intensities is shown as Percent Prepulse Inhibition. The average startle amplitude across all trials on which the startling stimulus was presented alone is shown as Startle Amplitude. Rats received an ICV infusion of either saline (5.0 µl) or mouse Ucn III (20.0 µg) once/day on each of 5 consecutive days. The rats were tested for PPI 2.5 h after the ICV infusion on days 1, 3, and 5. (a) Ucn III did not affect PPI on any of the test days. (b) There was an overall decrease in startle amplitude due to Ucn III, which was clearly due to effects on day 5.

4. Discussion

The present studies were designed to examine the effects of acute and repeated restraint stress on PPI in BN rats, and to determine whether the effect of restraint stress on PPI could be attenuated by a CRF_1 and/or a CRF_2 receptor antagonist. The major findings of the present studies were that 1) repeated, but not acute, restraint stress attenuated the increase in PPI caused by repeated PPI testing, and PPI was reduced by restraint following repeated PPI testing, 2) CRF_2 receptor blockade attenuated the effect of repeated restraint stress on PPI, but had no effect on restraint stress-induced changes in startle amplitude or on grooming behavior, 3) repeated ICV infusion of the selective CRF_2 receptor agonist, Ucn III, did not attenuate PPI on any test day, and 4) CRF_1 receptor blockade did not alter the effect of repeated restraint (or exogenous CRF) on PPI, startle amplitude, or grooming behavior, but did attenuate the anxiogenic effect of CRF. Although repeated restraint decreased PPI, restraint did not have acute effects on PPI, as exogenous CRF administration does.

In the first experiment, stress-naïve rats exhibited an increase in PPI over the 3 testing sessions of the 5-day experiment. This increase was significantly blunted in repeatedly restrained rats on day 3 and was marginally blunted on day 5. The second experiment

showed that restraint stress decreased PPI in rats exhibiting elevated levels of PPI due to repeated PPI testing prior to restraint exposure. Thus, restraint stress decreased PPI in addition to blunting the increase in PPI caused by repeated testing. This suggests that stress may alter sensorimotor gating in adult animals in two ways. Existing literature shows that early-life stress, particularly maternal separation and isolation-rearing, results in diminished PPI in adulthood (Weiss and Feldon, 2001). The effect of restraint stress on PPI in adult rodents has been examined in only a few studies to date and these findings are inconsistent. In one experiment, exposure to 1 or 11 days of 15–20 min restraint stress did not alter PPI (Acri, 1994; Faraday et al., 1999). In another experiment, 4 days of 2-h restraint exposure did not alter PPI, when PPI was assessed 3 weeks after restraint ended (Bijlsma et al., 2009). Exposure to 20-min of restraint for 3 weeks has been shown to decrease PPI on day 20 in male Long-Evans rats but not in male Sprague-Dawley rats (Faraday, 2002). Perhaps increasing the length of each restraint session beyond 20 min, or examining PPI soon after restraint termination, would have resulted in decreased PPI in the studies in which no effect of restraint was observed. It is evident that rat strain, number of days of restraint exposure, duration of each restraint session, and the time at which PPI is assessed post-restraint are important factors in the effects of restraint on PPI.

We used ASV-30 to examine whether CRF_2 receptor activation mediates the effect of restraint on PPI. As in our other experiments, 1 day of restraint did not affect PPI. On day 3 of restraint, there was an attenuation of the increase in PPI due to repeated testing and ASV-30 pretreatment did not alter this effect of restraint. However, on day 5 of stress, ASV-30 pretreatment blocked the effect of restraint on PPI. Although it is unclear why ASV-30 blocked the effect of restraint on PPI on day 5 but not on day 3, an intriguing possibility involves changes in CRF receptor localization following stress exposure. A recent study demonstrated that, in un-stressed rats, CRF_1 receptors were primarily localized to the plasma membrane while CRF_2 receptors were mainly in the cytoplasm of dorsal raphe nucleus neurons (Waselus et al., 2009). Interestingly, 24 h after swim stress, this localization changed and CRF_1 receptors became more apparent in the cytoplasm while CRF_2 receptors were localized to the plasma membrane (Waselus et al., 2009). In locus coeruleus neurons, swim stress caused CRF_1 receptor internalization which was apparent as early as 1 h after swim stress, with a greater effect at 24 h, highlighting the persistence of the internalization (Reyes et al., 2008). In another study, CRF_1 receptors were down-regulated in the cortex and hippocampus (CA1 and CA3) beginning 2 h, and lasting up to 24 h, after the termination of one 30-min restraint period (Greetfeld et al., 2009). In the study by Greetfeld et al., reductions in CRF_1 receptor binding were evident for up to 7 days post-stress, while CRF_2 receptors were up-regulated in the cortex at 4 and 24 h post-stress and in the hippocampal dentate gyrus beginning 24 h after stress. Thus, in our studies, it is possible that CRF_2 receptors were mainly localized to the cytoplasm during the initial days of restraint stress. As the days of restraint continued, CRF_2 receptors may have become present at the plasma membrane in sufficient numbers that ASV-30 binding could modulate behavior and attenuate the effect of restraint stress on PPI. Thus, dynamic stress-induced changes in CRF_1 and CRF_2 receptor localization at the plasma membrane may explain why repeated restraint affects PPI while acute restraint has no effect, and why several days of restraint are required for CRF_2 receptor blockade to affect behavior.

The fact that ASV-30 attenuated the effect of restraint on PPI suggests the possibility that Ucn II or Ucn III, peptides that have a selective affinity for the CRF_2 receptor, rather than CRF, may have mediated the effect of repeated restraint on PPI. In light of this

possibility, we examined the effect of repeated ICV infusion of Ucn III on PPI. We found that infusion of this peptide had no effect on PPI on any test day. These results suggest that activation of the CRF_2 receptor alone is not sufficient to produce effects on PPI that mimic those produced by CRF or stress. A second possibility is that Ucn III effects at the CRF_2 receptor are mediated by different signal transduction pathways than the effects of either CRF or stress (Brar et al., 2004; Hauger et al., 2006). It is possible that Ucn III would have decreased PPI had the rats undergone repeated restraint prior to infusion. This possibility will be tested in future experiments.

It is also possible that ASV-30 blocked the effect of restraint on PPI on day 5, but not on day 3, because neurotransmitters other than CRF may have initially mediated the effect of restraint on PPI. Since norepinephrine (NE) is released in response to restraint (Carrasco and Van de Kar, 2003), decreases PPI (Alsene et al., 2006; Carasso et al., 1998), and stimulates the release of CRF (Plotsky, 1987; Szafarczyk et al., 1987), it is possible that NE mediated the effect of restraint on PPI on day 3. As the days of restraint continued, an NE-induced release of CRF (Berridge and Dunn, 1989) may have allowed ASV-30 to attenuate the effect of restraint on PPI on day 5. It may also be that serotonin (5-HT) played a role in the delayed effect of ASV-30 on restraint stress in our study. In rats, acute restraint stress increases 5-HT release in the central nucleus of the amygdala and this effect is blocked by pretreatment with a non-selective CRF receptor antagonist, suggesting that stress-induced 5-HT release is mediated by central CRF receptor activation (Mo et al., 2008). Additionally, CRF_2 receptors are up-regulated in the dorsal raphe nucleus, a primary site of forebrain-projecting sero-tonergic neurons (Jacobs and Azmitia, 1992), in response to both acute (Waselus et al., 2009) and chronic (Lukkes et al., 2009) stress exposure. Anatomically, the central nucleus of the amygdala is one of the major sources of CRF innervation to the dorsal raphe nucleus (Gray, 1993) and the dorsal raphe nucleus provides 5-HT innerva-tion to the central nucleus of the amygdala (Petrov et al., 1994). Thus, it is possible that repeated restraint stress increased 5-HT release in the central nucleus of the amygdala, which caused sufficient up-regulation of CRF_2 receptors in the dorsal raphe nucleus by day 5 of restraint such that ASV-30 was able to effec-tively antagonize the CRF_2 receptors on this day. However, prior to day 5 of restraint, 5-HT itself may have been responsible for the restraint-induced decrease in PPI, as drugs that cause 5-HT release have been shown to reduce PPI (Kehne et al., 1996; Mansbach et al., 1989; Martinez and Geyer, 1997).

To our knowledge, these are the first studies to examine whether and which CRF receptor mediates the effect of restraint stress on PPI in rats. We demonstrated that CRF_2 receptor activation appeared to mediate the effect of repeated restraint stress on PPI in BN rats under the parameters used in this study, while CRF_1 receptor activation did not mediate this effect of stress. In 2004, Risbrough et al. showed that the CRF_1 receptor mediated a CRF-induced decrease in PPI in mice. It has also been shown that pretreatment with a selective CRF_1 receptor antagonist significantly increases PPI in CRF over-expressing mice (Groenink et al., 2008). In addition to the fact that mice were used in this study, it is possible that chronic over-expression of the transgene caused a develop-mental abnormality or adaptation of the CRF receptor system. In our studies, the CRF_1 receptor antagonist did not attenuate the effect of exogenously administered CRF in rats. More studies are needed to determine whether the differences between the results of our studies and those of others are due to species effects (mice vs. rats), or are peculiar to the BN rats used here. Future studies will examine how pretreatment with selective CRF receptor antagonists affect the restraint-induced decrease in PPI in other rat strains such as the WKY rats, which are less sensitive to the effects of both exogenous CRF and stress than BN rats (Conti, 2005; Conti et al.,

2002, 2009; Sutherland et al., 2008, 2010). These studies will reveal whether our findings generalize to other rat strains or whether our findings are unique to BN rats, which could perhaps make the BN rat a model for dysfunctional behavioral responses upon stress-induced activation of the CRF system. Overall, exam-ining additional rat strains would greatly improve our under-standing of how stress affects sensorimotor gating via the endogenous CRF system. Since 2.5 h elapsed between the onset of restraint stress and PPI testing in our experiment, a second possi-bility for the lack of an effect of CP-154,526 is that we were assessing PPI at a time-point when CRF_1 receptors were down-regulated and/or internalized such that CP-154,526 could not have altered behavior.

Although CP-154,526 did not attenuate the effect of CRF on PPI in our study it did attenuate the anxiogenic effect of CRF in the elevated plus maze, as previously shown by Zorrilla et al. (2002). Thus, CP-154,526 functioned effectively as a CRF_1 receptor antag-onist in our hands and the lack of an effect of the drug in the PPI experiments was not due to improper preparation or injection of the compound, ineffective dosing, or choosing a sub-optimal pretreatment period. It is possible that repeated stress altered CRF_1 receptor dynamics such that the antagonist was ineffective in the PPI experiments. Nevertheless, in these experiments, the CRF_1 receptor antagonist also did not attenuate the effect of acute CRF even though the antagonist was administered prior to the agonist.

In these experiments, neither ASV-30 nor CP-154,526 affected baseline startle or the CRF- or stress-induced decrease in startle amplitude. Since we have previously shown that repeated restraint stress decreases startle amplitude in some recombinant inbred rat strains with a BN and a Spontaneously Hypertensive Rat progenitor (Conti and Printz, 2003) and that ICV infusion of CRF decreases startle amplitude in BN rats (Sutherland et al., 2008), the effect of both restraint stress and CRF infusion on startle amplitude in BN rats is not unique to these experiments. Typically, however, CRF infusion increases startle amplitude in WKY and Sprague-Dawley rats (Conti et al., 2002; Jones et al., 1998; Liang et al., 1992; Schulz et al., 1996; Swerdlow et al., 1986; Walker et al., 2009), and this effect is blocked by pretreatment with a selective CRF_1 receptor antagonist (Schulz et al., 1996; Walker et al., 2009). In mice, pretreatment with either a CRF_1 or CRF_2 receptor antagonist attenuates the CRF-induced increase in startle amplitude, demon-strating that the two CRF receptors act in concert to mediate this effect of CRF (Risbrough et al., 2003). However, transgenic mice over-expressing CRF show decreased startle amplitude compared to wild-type controls (Dirks et al., 2002). Thus, stress-induced (Conti and Printz, 2003) and CRF over-expression-induced (Dirks et al., 2002) reductions in startle amplitude have been previously observed and BN rats are not unique in this feature. It is possible that CRF_1 receptors mediate stress- and CRF-induced increases in startle amplitude but not stress- and CRF-induced decreases in startle amplitude.

In rats, both stressful stimuli (Dunn et al., 1987; Spruijt et al., 1992) and ICV infusion of CRF (Dunn et al., 1987; Jones et al., 1998; Lazosky and Britton, 1991) have been shown to increase grooming behavior. In our studies, the restraint stress-induced increase in grooming behavior was not affected by either CP-154,526 or ASV-30 pretreatment, indicating that this effect of restraint was not mediated by activation of either CRF receptor type alone in BN rats or that blockade of both receptors is required to reduce the effect of stress on grooming. We also showed that CP-154,526 pretreatment did not alter the CRF-induced increase in grooming behavior, even though it attenuated a CRF-induced increase in anxiety-like behavior in the elevated plus maze. Our findings are in contrast to previous findings that a CRF_1 receptor antagonist attenuates stress-induced (Hotta et al., 1999) and

CRF-induced increases in grooming behavior (Howard et al., 2008). Although we did not examine the effect of CRF_2 receptor blockade on the CRF-induced increase in grooming, it is possible that CRF_2 receptors mediate this effect of CRF in BN rats. However, treatment with a CRF_2 receptor agonist does not increase grooming behavior in Sprague-Dawley rats (Howard et al., 2008). Since we observed grooming after a 2-h restraint period, it is possible that changes in CRF receptor localization have already occurred, such that CRF_1 receptors have become internalized while CRF_2 receptors are localized to the plasma membrane (Waselus et al., 2009). Thus, we may have assessed grooming behavior at a time when the main CRF receptor that is present at the plasma membrane is the CRF receptor that does not appear to mediate grooming behavior (Howard et al., 2008).

In conclusion, our studies demonstrate that restraint stress decreases PPI, in addition to blunting the increase in PPI caused by repeated testing, in BN rats. CRF_1 receptor activation does not appear to mediate the effect of repeated restraint (or exogenous CRF) on PPI, startle amplitude, or grooming behavior under the experimental parameters used here. CRF_2 receptor activation did, however, mediate the effect of repeated restraint stress on PPI in these experiments. It would be interesting to examine whether CRF_1 and CRF_2 receptors mediate stress and/or CRF effects on PPI at different times post-treatment, given the dynamic effects of stress and CRF on CRF receptor localization. These findings highlight the effect that stress can have on adult animals' information processing abilities, and elucidate a possible mechanism for the effect of restraint stress on PPI. Our studies, along with future studies aimed at understanding the mechanisms by which stress affects sensorimotor gating in more detail, may have important clinical applications for informing new treatments for people afflicted with psychiatric disorders in which information processing deficits are a hallmark.

Acknowledgments

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