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ENDURING SENSORIMOTOR GATING ABNORMALITIES FOLLOWING PREDATOR EXPOSURE OR CORTICOTROPIN-RELEASING FACTOR IN RATS: A MODEL FOR PTSD-LIKE INFORMATION PROCESSING DEFICITS?

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Abstract

A deficit in prepulse inhibition (PPI) can be one of the clinically observed features of post traumatic stress disorder (PTSD) that is seen long after the acute traumatic episode has terminated. Thus, reduced PPI may represent an enduring psychophysiological marker of this illness in some patients. PPI is an operational measure of sensorimotor gating and refers to the phenomenon in which a weak stimulus presented immediately before an intense startling stimulus inhibits the magnitude of the subsequent startle response. The effects of stress on PPI have been relatively understudied, and in particular, there is very little information on PPI effects of ethologically relevant psychological stressors. We aimed to develop a paradigm for evaluating stress-induced sensorimotor gating abnormalities by comparing the effects of a purely psychological stressor (predator exposure) to those of a noiceptive physical stressor (footshock) on PPI and baseline startle responses in rats over an extended period of time following stressor presentation. Male Sprague-Dawley rats were exposed (within a protective cage) to ferrets for 5 min or left in their homecage and then tested for PPI immediately, 24h, 48h, and 9 days after the exposure. The effects of footshock were evaluated in a separate set of rats. The effects seen with stressor presentation were compared to that elicited by corticotropin-releasing factor (CRF; 0.5 and 3μg/6μl, intracerebroventricularly). Finally, the effects of these stressors and CRF administration on plasma corticosterone were measured. PPI was disrupted 24h after ferret exposure; in contrast, footshock failed to affect PPI at any time. CRF mimicked the predator stress profile, with the low dose producing a PPI deficit 24h after infusion. Interestingly, the high dose also produced a PPI deficit 24h after infusion, but with this dose, the PPI deficit was evident even 9d later. Plasma corticosterone levels were elevated acutely (before PPI deficits emerged) by both stressors and CRF, but returned to normal control levels 24h later, when PPI deficits were present. Thus, predator exposure produces a delayed disruption of PPI, and stimulation of CRF receptors recapitulates these effects. Contemporaneous HPA axis activation is neither necessary nor sufficient for these PPI deficits. These results indicate that predator exposure, perhaps acting through CRF, may model the delayed-onset and persistent sensorimotor gating abnormalities that

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have been observed clinically in PTSD, and that further studies using this model may shed insight on the mechanisms information-processing deficits in this disorder.

1. INTRODUCTION

Post-traumatic stress disorder (PTSD) is one of the most devastating of the clinical sequelae of severe stress exposure, and is of great current concern given its high prevalence in veterans of the military engagements in Iraq and Afghanistan (Friedman et al., 2011; Ramchand et al., 2010; Ursano et al., 2010). One of the hallmark symptoms of PTSD is the remarkably low threshold for environmental stimuli to elicit exaggerated, negatively valenced affective responses such as fear and anxiety (Yehuda et al., 2006). In addition, it is well established that PTSD endures in time far beyond the acute precipitating stressor and is difficult to treat adequately (Yehuda and Bierer, 2009). Presently, the neural mechanisms underlying this profound, long-lasting change in affective processing are very poorly understood.

The enduring nature of changes in PTSD and the generalization of pathological responses to stimuli that are not unambiguously threatening could suggest that its etiology involves long-term plastic changes in neural circuits subserving basic stimulus processing. Accordingly, it has been repeatedly shown that PTSD patients (including combat veterans) display significantly augmented acoustic startle responses (Charney, 2003; Davis et al., 2010; Grillon et al., 1998a; Pitman et al., 1999; Stam, 2007; Yehuda, 2004), which has been modeled elegantly with systematic studies of fear conditioning and extinction in animals (Davis et al., 2008; Fanselow and Poulos, 2005; Myers and Davis, 2007; Rauch et al., 2006; Rodrigues et al., 2009).

Another important realm in which PTSD patients show abnormalities is in information processing mechanisms relevant to sensory or sensorimotor gating, as measured in several paradigms including prepulse inhibition (PPI) of the startle response (Clark et al., 2009; Ghisolfi et al., 2004; Gillette et al., 1997; Grillon et al., 1998b; Grillon et al., 1996; Holstein et al., 2010; Neylan et al., 1999; Ornitz and Pynoos, 1989; Skinner et al., 1999; Stewart and White, 2008). PPI refers to the ability of a weak pre-stimulus to attenuate the startle response to a superthreshold stimulus presented immediately after the pre-stimulus, and is one of the most widely used operational measures of sensorimotor gating in rodent models (Hoffman and Ison, 1980; Ison and Hoffman, 1983). PPI is thought to reflect the function of a pre-attentional sensory buffer, in which the processing of an environmental stimulus is “defended” from interference by other impinging stimuli for a brief period of time (Geyer, 2008). The breakdown of PPI has been proposed as a core endophenotype of several psychiatric illnesses, notably schizophrenia and Tourette’s syndrome, which have in common an inability to “filter out” competing, intrusive cognitive schemas or motor responses (Braff et al., 2008; Swerdlow et al., 2008). It is noteworthy in this regard that PTSD has been hypothesized also to include some forms of altered cognitive functioning (Bremner, 2006; Buckley et al., 2000; Horner and Hamner, 2002) including comorbid psychosis-like symptoms, a cognitive change seen also in schizophrenia (Lindley et al., 2000; Seedat et al., 2003). Hence, a breakdown of PPI could represent one of several enduring, coordinated changes in stimulus processing which promote the dysfunctional responses seen in PTSD.

Despite the existence of PPI deficits in PTSD patients, there are few animal models for these types of information-processing deficits in response to trauma. Particularly important in this regard is the utilization of stimuli that are ethologically relevant and also simulate in rodents the intensity of the PTSD-inducing traumatic experiences. Such stimuli would perhaps

produce effects distinguishable from more commonly used laboratory stressors, which acutely in adulthood have had inconsistent or no effects on PPI (Acri, 1994; Faraday et al., 1999; Liu et al., 2011; Sutherland et al., 2010; Sutherland and Conti, 2011). Hence, we examined an ethologically valid model of acute psychological trauma, in which rats are exposed to a ferret (a natural predator for rats) but protected from injury by a protective cage. It has been shown that this type of predator exposure elicits acute hypothalamic-pituitary-adrenal (HPA) axis activation along with defensive-like behaviors such as freezing and ultrasonic vocalizations (Blanchard et al., 1991; Roseboom et al., 2007). This model has considerable validity for the typical inducing stimuli of PTSD, and has been found to produce lasting anxiety-like responses in mice (Adamec et al., 2010; Adamec et al., 2008; Blanchard et al., 2001; Neumann et al., 2011).

Our main objective was to determine the effect of predator stress upon startle and PPI and to map out the time-course of these putative effects, because no information exists regarding the longer term effects of predator exposure on PPI. In the course of these experiments, it was found that predator stress produces a delayed disruption of PPI. We then explored the behavioral specificity of this effect, and compared the profile of results to that elicited by directly stimulating one of the main central mediators of stress, the corticotropin-releasing factor (CRF) system (Bale and Vale, 2004). Moreover, because PPI deficits can be produced with increased CRF transmission (Conti et al., 2002; Dirks et al., 2002; Risbrough et al., 2004; Tejeda et al., 2010), we examined how stress- and CRF-induced effects mapped onto activation of one classic stress-responsive system: the hypothalamic-pituitary-adrenal (HPA) axis (Jankord and Herman, 2008).

2. MATERIALS AND METHODS

2.1: Animals

One-hundred and thirty-one male Sprague-Dawley rats (Harlan Laboratories, Madison WI) weighing 300-400g were housed in pairs in clear cages with *ad libitum* access to food and water in a light- and temperature-controlled vivarium, and were maintained under a 12-h light/dark cycle (lights on at 0700). All facilities and procedures were in accordance with the guidelines regarding animal use and care from the NIH of the USA, and were supervised and approved by the Institutional Animal Care and Use Committee of the University of Wisconsin.

2.2: Surgery

Rats were anesthetized with ketamine/xylazine (80mg/12mg per ml; Phoenix Scientific, St. Joseph, MO), and secured in a stereotaxic frame (Kopf Instruments, Tujunga, CA). Stainless steel cannulae (23-gauge, Small Parts, Miami Lakes, FL) were implanted and affixed to the skull with dental cement (Lang Dental Mfg, Wheeling, IL) and anchoring skull screws (Plastics One, Roanoke, VA) and were aimed at the lateral ventricle using the atlas of Paxinos and Watson (1998). Final coordinates in mm from bregma were: -1.0 (AP); ± 1.4 (LM; the laterality of the lateral-medial coordinate was alternated between rats); -2.1 from skull surface (DV). Wire stylets were placed in the cannulae to prevent blockage, and rats recovered for a week before testing.

2.3: Drugs and Microinfusions

Corticotropin-releasing factor was obtained from Bachem/Peninsula Labs (Torrance, CA) and dissolved in sterile distilled water. Doses were calculated as salts based on previous studies indicating potent behavioral effects with ICV infusion (Koob, 1999). For microinfusions, stylets were removed and cannulae were cleaned with a dental broach (Henry Schein, Melville, NY); stainless steel injectors (30-gauge, Small Parts, Miami Lakes,

FL) were lowered to extend 2.0 mm past the tip of the cannula. Injectors were attached with polyethylene tubing (PE-10, Becton Dickinson, Sparks, MD) to 10- μ l glass Hamilton syringes (Hamilton Co., Reno, NV). Infusions were delivered by manual depression of plungers at a rate of 6 l over 10 sec. After infusions, injectors were left in place for 1 min to allow for absorption of the solution before replacement of stylets.

2.4: Startle Chambers

Startle chambers (San Diego Instruments, San Diego, CA) contained a nonrestrictive Plexiglas cylinder resting inside a ventilated and illuminated sound-attenuating cabinet, with a high-frequency loudspeaker to produce all acoustic stimuli. As described previously (Mansbach et al., 1988), the whole-body startle response of the animal caused vibrations which were then converted into analog signals by a piezoelectric unit attached to the platform. These signals were digitized and stored by a microcomputer and interface unit. Monthly calibrations were performed on the chambers to ensure accuracy of the sound levels and measurements. Sound levels were measured using the dB(A) scale.

2.5: Startle and PPI Testing

The test session consisted of a background noise (65 dB) that was presented alone for 5 min and remained on for the length of the session, followed by presentation (in a pseudo-random order) of Pulse-Alone trials (40-msec, 120-dB broadband bursts), Prepulse + Pulse trials (20-msec noises that were 3, 6, or 12 dB above the background noise and were presented 100 msec before the onset of the 120-dB pulse), and No Stimulus trials (only the background noise). The session contained 52 trials (20 Pulse-Alone trials and eight each of the remaining trial types) presented in a pseudorandom order; an average of 15 sec separated consecutive trials. Four Pulse-Alone trials were also presented at the beginning of the session to ensure that startle magnitude was stable during the portion of the session when PPI was measured, as the most rapid habituation of the startle response occurs within the first several presentations (Geyer et al., 1990); these 4 Pulse-Alone trials were excluded from the calculations of startle and %PPI. During the week before the drug testing began, all rats underwent one exposure to the startle test session per day on 3 separate days; for rats in the ICV infusion experiments, sham infusions were performed prior to the last test to familiarize rats with the testing and microinfusion procedures prior to the commencement of drug testing.

2.6: Blood sampling and corticosterone RIA

Rats with chronic indwelling jugular catheters were purchased from Harlan Laboratories (Madison, WI) for Experiment 5 (see below). Briefly, access to the catheter was provided by a 4-cm length of tubing that protruded from the nape of the neck, and that was plugged with a stainless steel wire plug. Blood samples were collected by removing the plug, inserting a 1-cc syringe with a 26-gauge needle into the externalized tubing, and withdrawing approximately 0.3 ml of blood. Catheters were then flushed with a 0.25 ml of sterile isotonic saline followed by 0.1 ml of a 500-U/ml heparinized glycerol solution. This same saline and heparin/glycerol flushing procedure was used every day to check and maintain catheter patency throughout the experiment. Rats in this experiment that also received ICV infusions underwent stereotaxic surgery upon arrival (and thus were equipped with both a jugular catheter and an ICV cannula); these rats were allowed a one-week post-surgical recovery period before testing began.

Plasma was prepared by centrifugation at $2,000 \times g$ for 10 min at 4 °C and stored at 80°C. Plasma corticosterone was measured with an enzyme immunoassay kit (Diagnostic Systems Laboratories, Webster, TX) that has a lower limit of detection, defined as the concentration of the lowest standard included in the assay, of 5 ng/ml. The inter-assay coefficient of

variation (CV) was 10.0% and the average intra-assay CV was 5.8%. Samples were assayed in duplicate and results were accepted only if the CV% was < 20%.

2.7: Experimental Design

For all experiments involving PPI/startle testing, all rats underwent three baseline startle/PPI tests one week after arrival in order to acclimate them to the testing procedure. For rats undergoing stereotaxic surgery (Experiments 3 and 4), these baseline tests occurred one week after recovery from surgery. Two days after baseline testing, experimentation began.

2.7.1. Experiment 1: Predator stress effects on PPI and startle—Separate groups of experimentally naïve rats were assigned randomly into either a trauma group (Ferret , N = 9) or a control group (No Stress , N = 12). Rats in the Ferret group were placed individually in a protective metal wire cage (7" × 8" × 9") that was secured to the floor of the homecage of a ferret for 5 min. This procedure allowed animals to see, hear, and smell each other, but did not permit direct physical contact. Rats in the No Stress group remained in their homecages during this time in a separate room. Upon completion of these procedures, all rats were brought to the testing room and tested for PPI; the rats were re-tested 24h, 48h, and 9d later using the same test session (but with no additional ferret exposure).

2.7.2. Experiment 2: Footshock effects on PPI and startle—Separate groups of experimentally naïve rats were assigned randomly into either a stress group (Footshock', N = 8) or a control group (No Stress , N = 9). Rats in the Footshock group received 3, 1.5 mamp, 1-sec footshocks (consecutive shocks separate by 20 sec) via a wire grid floor in a Gemini shuttlebox (San Diego Instruments, San Diego, CA); control (No Stress) rats remained in their homecages during this time in a separate room.. Five min later, all rats were brought to the testing room and tested for PPI; the rats were re-tested 24h, 48h, and 9d later using the same test session (but with no additional stress exposure).

2.7.3. Experiment 3: High-dose CRF effects on PPI and startle—In order to determine the time course for optimal CRF-induced behavioral effects for the startle/PPI testing, first a cohort of rats (N = 8) was tested for grooming and exploratory behaviors following ICV infusions of CRF (3 g) or vehicle (dH₂O), as these are well-documented sequelae of ICV CRF administration. Thus, rats received either vehicle or CRF immediately before being placed individually in behavioral observation cages (identical to homecages and to which rats had been acclimated on the two previous days for 1h each). The frequency and duration of locomotion (cage crossings) and grooming behavior was rated by an experimenter blind to the rats' treatment condition for 1 h (analyzed as a time course in 3, 20-min blocks). Five days later, this procedure was repeated except that rats previously receiving CRF now received vehicle and vice versa. In separate cohorts of rats, preliminary analysis of acute CRF-induced effects on PPI were evaluated by comparing PPI in rats treated with either vehicle or 3μg CRF. Thus, one cohort (N = 5–6 per vehicle/CRF condition) was tested 15 min after infusions and another (N = 8 per vehicle/CRF condition) was tested 30 min after infusions.

Because no effects on PPI were seen with these initial 15- and 30-min post-infusion testing time points, and because maximal CRF-induced elevations in locomotion and grooming were seen between 40 and 60 min after infusion (Figure 3), a 45-min post-infusion testing time point was selected for the subsequent long-term startle/PPI testing to maximize the possibility that acute CRF-induced effects on PPI would be detected. In this experiment, separate groups of experimentally naïve rats were assigned randomly into either a high-dose CRF group (CRF 3 g , N = 7) or a control group (Vehicle , N = 8). Rats in the CRF 3 g

group received an ICV infusion of 3 g of CRF; control rats received an equivalent volume of dH₂O. Forty-five min later, all rats were brought to the testing room and tested for PPI; the rats were re-tested 24h, 48h, and 9d later using the same test session (but with no additional infusions).

2.7.4. Experiment 4: Low-dose CRF effects on PPI and startle—In order to determine if lower doses of CRF would produce similar effects, an additional experiment was conducted. Thus, separate groups of experimentally naïve rats were assigned randomly into either a low-dose CRF group (CRF 0.5 g , N = 8) or a control group (Vehicle , N = 6). Rats in the CRF 0.5 g group received an ICV infusion of 0.5 g of CRF; control rats received an equivalent volume of dH₂O. Forty-five min later, all rats were brought to the testing room and tested for PPI; the rats were re-tested 24h, 48h, and 9d later using the same test session (but with no additional infusions).

2.7.5. Experiment 5: Effects of treatments on plasma corticosterone levels—This experiment assessed if the same manipulations that altered PPI/startle responses also changed plasma corticosterone levels, which was used as an index of activation of the hypothalamic-pituitary-adrenal (HPA) axis. Experimentally naïve rats with chronic indwelling jugular catheters were divided randomly into the following groups: No Stress (N = 5); Ferret (N = 4); Footshock (N = 4); Vehicle (N = 5); CRF 0.5 g (N = 6); CRF 3 g (N = 5). Rats were exposed to the same stress/infusion procedures that are described above, and then at the exact time point corresponding to the occurrence of the startle/PPI testing in the above experiments, instead had a blood sample withdrawn from the jugular catheter (equipped with an external port). Thus, blood samples were taken immediately after the stress procedures (No Stress , Ferret and Footshock groups) or 45 min after ICV infusions (Vehicle and CRF groups). Twenty-four hours later, one additional blood sample was taken from all rats to compare acute and 24h plasma corticosterone time points.

2.8: Verification of ICV infusions

At the end of all infusion experiments, rats were given an overdose of sodium pentobarbital (Abbott Laboratories, North Chicago, IL) and given an ICV infusion of 5 µL of Chicago Sky Blue Dye (Sigma, St. Louis, MO). After infusion of the dye, rats were decapitated and brains were sliced into 1-mm sections. If dye was observed in the 3rd and 4th ventricles (indicating spread throughout the ventricular system from the original infusion site in the lateral ventricle), rats were considered to have accurate ICV infusions cannula placements. Placements were verified by an experimenter blind to the behavioral data; rats with missed placements were excluded from subsequent behavioral analyses. Sample sizes for each experiment reflect this final adjusted number.

2.9: Data Analysis

The startle response to the onset of the 120-dB burst was recorded for 100 msec for each Pulse-Alone, Prepulse+Pulse, and from the onset of each No Stimulus trial. Two measurements (startle magnitude and PPI) were calculated from these values for each rat for each of the different treatment conditions. Startle magnitude was the average of the startle responses to all Pulse-Alone trials. PPI was calculated as a percent score for each Prepulse + Pulse trial type: $\% \text{ PPI} = 100 - \{[(\text{startle response for Prepulse} + \text{Pulse trial})/(\text{startle response for Pulse-Alone trial})] \times 100\}$. Startle magnitude and data were analyzed with two-factor ANOVAs with test day as a within-subjects factor and treatment as a between-subjects factor; PPI data were analyzed similarly with three-factor ANOVAs with prepulse intensity as an additional repeated measure. When significant test day $\times$ treatment interactions were observed, analysis of simple main effects at each individual test day were conducted. Locomotor activity data (Experiment 3) were analyzed with separate 2-factor

ANOVAs for each activity measure, with CRF treatment and time (min post infusion) as within-subjects factors. For the corticosterone data, the ICV infusion groups and the stress groups were analyzed in separate 2-factor ANOVAs (each with treatment as a between-subjects factor and test day as a repeated measure) so that each type of manipulation was compared to its most appropriate control (i.e., CRF groups to a vehicle infusion group and stress groups to a no-stress group). *Post-hoc* analyses were done using Newman-Keuls tests. The alpha level for significance was set at 0.05.

3. RESULTS AND DISCUSSION

For every experiment, a significant main effect of prepulse intensity was seen upon analyzing %PPI data (F-ratios ≥ 39 , P-values 0.001); this is a standard parametric feature of PPI in which increasing prepulse intensities elicit higher levels of PPI (Braff et al., 2001). For the sake of brevity, this main effect is not repeated throughout the Results section. Unless indicated specifically otherwise, there also were no prepulse intensity $\times$ treatment interactions.

3.1: Ferret exposure disrupts PPI

As shown in Figure 1, exposure to the ferret resulted in a significant disruption of PPI 24h after stressor presentation. Omnibus ANOVA indicated no significant main effects of stress condition [$F(1,19)=0.7$, NS] or test day [$F(3,57)=1.5$, NS] for PPI, but did reveal a significant 3-way interaction between stress condition, test day, and prepulse intensity [$F(6,114)=3.0$, $P<0.009$]. Subsequent analyses indicated a significant effect of stress condition at 24h after stress [$F(1,19)=7.3$, $P<0.02$], with PPI values for ferret-exposed rats significantly lower than those for the No Stress group at the 6-dB ($P<0.01$) and 12-dB ($P<0.05$) prepulse intensities (Figure 1B). There was also a prepulse intensity $\times$ stress condition interaction at the 9d test day [$F(2, 38)=7.3$, $P<0.002$], which post-hoc tests revealed was due to a small increase in PPI levels in ferret-exposed rats compared to No Stress controls ($P<0.05$) at the 3-dB prepulse intensity on this test day (Figure 1D). No significant effects were seen on any of the other test days.

3.2: Footshock does not alter PPI

In contrast to the predator (ferret) stress, footshock failed to alter either PPI or baseline startle at any test day (acute, 24h, 48h, or 9d) after stress (Figure 2). There was no significant main effect on PPI of stress condition [$F(1,15)=0.2$, NS] or test day [$F(3,15)=1.2$, NS]. There were also no significant test day $\times$ stress interactions (all F ratios < 1.7 and P-values > 0.09).

3.3: CRF disrupts PPI

Several experiments with CRF were completed; these are shown in Figures 3–5. First, the effects of high-dose CRF (3 $\mu\text{g}/6 \mu\text{l}$) on measures of locomotor activity were assessed. ANOVAs showed a main effect of CRF treatment for cage crossings (locomotion) [$F(1,7)=18.7$, $P<0.004$] and grooming duration [$F(1,7)=16.5$, $P<0.005$], as well as a CRF treatment $\times$ time interaction for cage crossings [$F(2,14)=5.4$, $P<0.02$]. Subsequent analyses indicated that the 3- μg dose of CRF increased locomotion at the 21–40min ($P<0.05$) and 41–60min ($P<0.01$) post-infusion time points, and increased grooming at all three time points ($P<0.05$ –0.01) (Figure 3A–B). Thus, ICV CRF produced significant behavioral effects within the first hour after infusion. This same dose of CRF, however, failed to alter PPI at any of the acute post-infusion time points that were examined. There were no main effects of CRF treatment [$F(1,9)=0.1$, NS] or any prepulse $\times$ treatment interactions [$F(2,18)=0.6$, NS] in rats tested 15 min after infusions or 30 min after infusions {[$F(1,14)=0.1$, NS main effect of CRF treatment], and [$F(2,28)=0.5$, NS prepulse $\times$ treatment interaction]}; these results are

displayed in Figure 3C-D. Similarly, no effects of CRF were produced on baseline startle at either the 15-min or 30-min post-infusion time points (F -ratios <1.7 , P -values >0.2 ; data not shown).

Since the maximal CRF-induced behavioral effects were not evident until more than 40 min after infusion, one additional experiment was conducted to test for PPI effects 45 min after ICV CRF infusion; these rats were also re-tested 24h, 48h, and 9d later. In this case, a significant effect of CRF treatment was seen in the omnibus ANOVA [$F(1,13)=5.1$, $P<0.04$]. Subsequent analyses indicated that there was no change in PPI with CRF acutely (45 min post infusion) [$F(1,13)=0.1$, NS], but there was a significant effect 24h [$F(1,13)=4.7$, $P<0.048$], 48h [$F(1,13)=5.2$, $P<0.039$], and 9d [$F(1,13)=6.0$, $P<0.029$] later. Post-hoc comparisons revealed that CRF-treated rats had lower PPI levels than their vehicle-treated counterparts at multiple prepulse intensities ($P<0.05$) on each of these 3 later test days (Figure 4A-D). Thus, a high dose (3 μ g) of CRF produced a delayed-onset, but long-lasting deficit in PPI that was evident even 9 d after the infusion had taken place.

To determine if a lower dose of CRF would elicit a similar profile, one additional experiment was conducted with 0.5 μ g of CRF (Figure 5A-D). This dose of CRF did not affect PPI 45 min after infusion (acute test day) [$F(1,12)=0.02$, NS], but did decrease PPI 24h later [$F(1,12)=6.6$, $P<0.024$]; PPI values for the 0.5- μ g CRF dose group were significantly lower than those for the vehicle group at the 3-dB and 6-dB prepulse intensities on this test day ($P<0.05$). By 48h post infusion, there was no longer a main effect of CRF infusion [$F(1,12)=1.2$, NS]; the 9d test day also failed to show an effect of CRF infusion [$F(1,12)=1.6$, NS]. Thus, the lower dose of CRF disrupted PPI 24h after infusion, and this effect normalized by the 48h test day. This profile was therefore less robust than that of the high dose and was very similar to the profile of results produced by the predator stress.

3.4: PPI effects do not require concomitant alterations of baseline startle

Baseline startle responses were unaffected by ferret stress (Figure 6A) on any test day; no significant main effect of ferret stress [$F(1,19)=0.8$, NS] nor any significant interaction between ferret stress and test day [$F(3,57)=1.5$, NS] was seen. In the footshock experiment (Experiment Figure 6B), there were no main effects of stress condition [$F(1,15)=1.8$, NS] or test day [$F(3,15)=2.1$, NS] on startle, but there was an interaction between these factors [$F(3,45)=3.4$, $P<0.03$], resulting from the higher baseline startle values in the No Stress group compared to the Footshock group acutely (immediately after stress exposure) ($P<0.05$). In contrast, ICV infusion of the high dose of CRF (3 μ g) caused a sustained elevation in startle, as indicated by a significant main effect of CRF treatment in this experiment [$F(1,13)=4.9$, $P<0.045$]; the high-dose CRF group had higher startle values than vehicle-treated rats across the first three test days ($P<0.05$) (Figure 6C). There was no main effect of test day [$F(3,13)=2.1$, NS] or test day $\times$ treatment interaction [$F(3,39)=0.4$, NS]. The lower dose of CRF (0.5 μ g), however, had no effect on baseline startle responses (Figure 6D), as evidenced by the lack of a significant main effect of treatment [$F(1,12)=0.2$, NS] and the lack of a treatment $\times$ test day interaction [$F(3,36)=2.3$, NS]. In summary, it appears that alterations in startle magnitude can be dissociated from changes in PPI since some manipulations that decreased PPI (i.e., ferret stress, low-dose CRF) did not affect startle, and some that altered startle (i.e., footshock) did so without altering PPI.

3.5: Effects of ferret, footshock, and CRF on plasma corticosterone levels

Figure 7 shows the effects of the various treatments on plasma corticosterone levels, either acutely (to match the acute time point at which PPI testing had taken place with each of these manipulations) or 24h later (corresponding to when PPI deficits first emerged with ferret exposure and CRF infusions). ANOVA indicated a significant main effect of stress

condition [$F(2,10)=19.9$, $P<0.001$] and test day [$F(1,10)=32.8$, $P<0.001$], and an interaction between these factors [$F(2,10)=15.7$, $P<0.001$]. Subsequent analyses showed that immediately after stressor presentation (the acute time point), both ferret stress and footshock significantly elevated plasma corticosterone levels above those of the No Stress group ($P<0.001$), but that at the 24h time point, there were no differences between the three groups in terms of this measure. Similarly, there was a significant main effect of CRF infusion [$F(2,13)=4.1$, $P<0.04$] and test day [$F(1,13)=42.5$, $P<0.001$], and a significant CRF $\times$ test day interaction [$F(2,13)=14.8$, $P<0.001$]. Post-hoc comparisons indicated that at the acute time point, both doses of CRF increased plasma corticosterone values above those of the vehicle infusion ($P<0.05$ – 0.01), but that levels for the 3- μ g dose were even higher than those for the lower dose ($P<0.05$). At the 24h time point, there were no differences between the groups. Thus, marked elevations in plasma corticosterone levels were produced by all the treatments acutely (when no PPI deficits were observed), but plasma corticosterone values returned to control levels 24h later, when PPI deficits were first seen with any treatment. Thus, predator stress- or CRF-induced PPI deficits were not contemporaneously associated with alterations in plasma corticosterone levels.

4. CONCLUSIONS

4.1: Summary of results

Several important results were obtained from the present studies. First, a significant reduction in PPI was observed 24h but not immediately after predator stress exposure. Thus, a stressor that may represent an ethologically valid animal model for psychological trauma (protected predator exposure) elicited a delayed-onset post-trauma disruption in sensorimotor gating, a fundamental form of information-processing that has been reported deficient in PTSD patients (Braff et al., 2001; Grillon et al., 1998b; Grillon et al., 1996). Second, central infusion of corticotropin-releasing factor (CRF), one of the most strongly implicated neuroendocrine mediators of stress responses (Bale and Vale, 2004; Binder and Nemeroff, 2010; Koob and Zorrilla, 2010; Valentino et al., 2010), also produced a PPI deficit 24h after infusion; the deficit induced by the highest dose of CRF (3 μ g) was present even 9d later. Thus, CRF caused a delayed and enduring PPI deficit that was present long after the termination of the immediate stimulus (infusion), similar to the PPI deficits seen in PTSD, which are detectable long after the acute traumatic event has ended. Finally, concomitant elevations in baseline startle magnitude or plasma corticosterone were neither necessary nor sufficient to produce PPI deficits in these studies, suggesting that the predator stress- or CRF-induced disruption in sensorimotor gating represented a distinct phenomenon that was not simply an artifact of either of these other measures. Taken together, the present results indicate that predator exposure or direct activation of central CRF systems can produce a profile of results that closely resembles the deficits in sensorimotor gating that have been reported in PTSD. Hence, these manipulations may represent viable approaches for modeling in rodents one of the fundamental types of information-processing deficits seen in these patients.

4.2: Comparison of effects with different stressors and ICV CRF

One interesting dissociation seen in the present studies was between predator stress and footshock, with the latter stimulus having no significant effects on PPI at any time point. The reason for this different profile after predator stress versus footshock is not clear. Both stressors were roughly equipotent in terms of the amplitude of acute corticosterone release. Nevertheless, the predator was a solely psychogenic stimulus (rats never had physical contact with the ferrets). In contrast, footshock involves a strong nociceptive element that could, for example, trigger compensatory neural substrates such as opiates to promote reactive analgesia (Urca et al., 1985; Watkins et al., 1982). As opiates and CRF produce

opposing effects in systems that subserve PPI (i.e., the locus coeruleus) (Bakshi and Alsene, 2010; Valentino and Van Bockstaele, 2001), it is possible that putative PPI-disrupting effects were canceled out by these opposing systems that may have been concomitantly recruited by footshock. Indeed, these two stressors produce differential neuronal activation patterns. We have found that this same ferret stress procedure elicits significantly more FOS expression than footshock does in the medial amygdala, a site that recently also has been shown to regulate PPI (Cloninger et al., 2009; Vinkers et al., 2010). Further studies are needed to clarify these mechanisms, but it is tempting to speculate that a predator attack in rats could bear a closer resemblance (at least in terms of face validity) to life-threatening psychological trauma, and that this type of stress produces a unique effect on sensorimotor gating and neural activation.

4.3: Predator stress- and CRF-induced PPI deficits were delayed in their onset

The current results that predator stress and CRF disrupt PPI corroborate and expand the previous findings discovering that CRF disrupts sensorimotor gating (Conti et al., 2002; Dirks et al., 2002; Risbrough et al., 2004). In contrast to these prior results, PPI effects were not seen acutely in the present studies, despite the elicitation of potent locomotor-activating and neuroendocrine (corticosterone release) at this time point. The one previous study that examined predator stress effects on PPI also found that PPI was unaffected immediately (acutely) after mice were exposed to the odor of a (rat) predator (Duncan et al., 2004). To the best of our knowledge, the present results are the first to indicate that predator stress alters PPI, and that there can be a *delayed* emergence of PPI deficits (24h later) after CRF or stress challenge. Several possible factors could contribute to the different time course profiles observed in the present studies versus in those earlier reports. For example, species and strain differences that already are known to factor prominently into the relative efficacy of CRF to disrupt PPI acutely also could influence whether deficits emerge immediately or after a delay, as the current subjects were Sprague-Dawley rats whereas the previous CRF studies in rats were conducted in other strains (Conti, 2005; Conti et al., 2002). It may be that underlying differences in the genetic make-up of these strains mediate differential sensitivity to CRF-induced PPI deficits, just as individual differences between animals influence anxiety-related responses (Cohen et al., 2003; Qi et al., 2010) and in humans determine the propensity for developing PTSD after trauma exposure (Stam, 2007; Yehuda, 2004). Similarly, PPI deficits have not been detected in certain clinical cohorts with PTSD (Grillon et al., 1998; Holstein et al., 2010; Lipschitz et al., 2005), although it was suggested that methodological issues including the failure to sort subjects by menstrual cycle phase, which significantly affects basal PPI levels (Swerdlow et al., 1997), may have contributed to some of these null findings.

The present findings for the first time indicate the conditions under which delayed effects can be produced on PPI with predator stress and CRF. This delayed time course potentially may be an important feature for the modeling of information filtering abnormalities in PTSD, since in this illness, PPI disruption and other sensorimotor gating deficits also exist long after the acute (traumatic) stimulus has terminated. Another potential clinical implication of the current findings may be in the realm of schizophrenia, which is perhaps the best-characterized psychiatric illness in which PPI deficits are manifested (Braff et al., 2008; Geyer, 2008; Swerdlow et al., 2008). It is well known that schizophrenia symptomatology can be exacerbated or triggered by intense stress that is often of a psychological/emotional nature (Betensky et al., 2008; Horan et al., 2005; Nuechterlein et al., 1994; Walker and Diforio, 1997). Thus, it is possible that the gradual worsening of symptoms in schizophrenia and PTSD that occurs in the days and weeks after the triggering event could reflect a delayed-onset deterioration of PPI such as that seen in the present study. Interestingly, delayed progressive worsening of PPI has been seen in humans,

putatively in response to repeated psychological stress (Grillon and Davis, 1997). Moreover, a tendency for reduced PPI has also been seen in non-PTSD veterans who had been exposed to trauma (combat) (Grillon, et al., 1998b).

Defensive behavior (particularly to signals indicative of predators) has been hypothesized to follow a bi-modal time course, with focused attention to the extant threat acutely, and distributed attention (scanning the environment) after the threat has ended (Blanchard and Blanchard, 1989). Consistent with the notion that focused processing of signals associated with actual threat may be adaptive, it has been shown that acute threat may actually improve sensorimotor gating (Cornwell et al., 2008; Grillon and Davis, 1997). Such mechanisms could explain why the literature on the effects of stress on PPI is so mixed, with stress sometimes improving and sometimes disrupting PPI, with a great dependence on the type and duration of the stressor, the gender and age of the subjects during stress exposure, and the post-stress timing of the PPI testing (Chester et al., 2008; Choy et al., 2009; Ellenbroek et al., 1998; Faraday, 2002; Heldt and Ressler, 2006; Koenig et al., 2005; Le Pen et al., 2006; Pijlman et al., 2003; Powell and Geyer, 2002; Sutherland et al., 2010). Sensorimotor gating has been conceptualized as a mechanism that has the effect of potentially filtering out intrusive stimuli, thereby possibly defending higher-order attentional control (Braff et al., 2008; Geyer, 2008; Hetrick et al., 2011; Swerdlow et al., 2008). Given that stress-induced performance decrements on selective-attention tasks may stem from “attentional lability” associated with diffuse attention to multiple targets (Aston-Jones and Cohen, 2005; Berridge and Waterhouse, 2003; Foote et al., 1980; Robbins and Arnsten, 2009), it is conversely possible that low sensorimotor gating (i.e., disrupted PPI) is associated with a state in which a broader range of stimuli (albeit less completely processed) gain access to higher order cognitive domains and thereby facilitate attentional flexibility in the aftermath of a traumatic event. In other words, a *decrement* in focused attention may correspond to an *improvement* in rapid shifting among multiple ambiguous targets, which may be adaptive in certain situations. Whether diminished PPI is a cause, result, or even a correlate of changes in higher-order stimulus processing is not clear. Nevertheless, we speculate that the PPI disruption 24h after predator exposure could theoretically correspond to the organism having shifted into a low-PPI / high-scanning mode, which has been proposed as a means to enhance survival in a post-threat environment that could potentially be dynamic, unpredictable, and require attentional and behavioral flexibility (Blanchard and Blanchard, 1989). Thus, a delayed post-stress deficit in PPI could represent a condition in which reduced PPI is actually an adaptive change.

In PTSD, perhaps the individual has been shifted into the scanning/vigilant mode for an enduring period of time, possibly stemming from a sensitization of the neural systems mediating these processes, and this is what the persistent PPI deficits in these patients may indicate. In this case, because the actual threat is *far* removed (with no imminent danger), the persistence of a low-gating (reduced PPI) mode may be maladaptive insofar as it could favor the entry of multiple innocuous signals into the processing spectrum, that under high-gating conditions might be filtered out. To the best of our knowledge, the present results are the first to demonstrate such a long-lasting (9d post infusion) PPI disruption following a single administration of CRF; the high dose (3 g) was unique in this regard, as lower doses (0.5 g) and a single 5-min presentation of predator stress caused similar delayed-onset PPI disruptions, but that normalized by this later test day. Thus, it is tempting to speculate that there may be a dose-effect function for stress/CRF effects on PPI, with larger amounts producing greater impairment that are longer lasting. Thus stronger and more repeated trauma presentations might cause more enduring changes in the systems mediating these PPI disruptions. Perhaps this is why the high-dose CRF effects on PPI were the longest lasting. Accordingly, repeated, but not acute, restraint stress disrupts PPI via a CRF-mediated mechanism in rats, and repeated, but not acute, CRF receptor stimulation in portions of the

amygdala disrupts PPI and produces enduring changes in anxiety-related behaviors (Bijlsma et al., 2011; Shekhar et al., 2005; Sutherland and Conti, 2011).

The neural mechanisms underlying this delayed and long-lasting profile of predator stress and CRF-induced PPI deficits are not clear at present. Actions within the locus coeruleus (LC) may be important, since it has been shown that stress and CRF shift LC into a high tonic discharge state, that CRF in LC enhances behavioral flexibility, and that pharmacological stimulation of LC with drugs that potently drive tonic firing of this nucleus disrupts PPI (Bakshi and Alsene, 2010; Valentino and Van Bockstaele, 2008; Valentino et al., 2010). These effects are, however, seen acutely and further experimentation is needed to see whether they last as long as the PPI disruptions seen presently. It is interesting to note that significant neuroplasticity in CRF-bearing neurons of the LC has been observed 24h after the (stress or CRF infusion) stimulus, but whether CRF receptor trafficking that occurs in response to stress or CRF infusion also shows a long-lasting (i.e., 9d) time course is not known (Reyes et al., 2006; Reyes et al., 2008). Clearly, additional experimentation is required to validate these hypotheses, but the current findings could represent an important heuristic framework for etiological studies of sensorimotor gating abnormalities in PTSD and schizophrenia, and also for the development of integrated theories on the relationship between pre-attentional sensory buffering and executive attentional control.

4.5: PPI effects were dissociable from alterations in baseline startle responses

The lack of effects on startle at first may seem to contradict the relevance of these findings for PTSD, since startle elevation is one of the most commonly cited features of PTSD and has been modeled in rats with footshock stress (Rasmussen et al., 2008). Nevertheless, the neural substrates for PPI and startle differ, and startle effects are not necessary or sufficient for producing PPI deficits (Swerdlow et al., 2001), including in PTSD patients (Grillon et al., 1998a; Grillon et al., 1996). In the present study the high dose of CRF produced a prolonged elevation in startle, although at 9d startle had normalized but the PPI deficit was still present. Again, this finding demonstrates that startle changes and PPI deficits are not dependent on each other and suggests that these could represent simultaneous parallel effects. Deficient PPI could therefore represent one aspect of PTSD, a disorder that may consist of multiple simultaneous pathologies. In this regard, it is important to note that in addition to the debilitating affective symptoms, PTSD patients also present with numerous cognitive problems that resemble schizophrenia symptoms that are often attributed to a breakdown in pre-attentional sensory filtering (Clark et al., 2009; Karl et al., 2006a; Orr et al., 2002; Stewart and White, 2008). Indeed, some of the circuits implicated in PTSD overlap with those thought to contribute to information-processing disturbances in schizophrenia (Bremner et al., 2008; Ghisolfi et al., 2004; Karl et al., 2006b; Liberzon and Sripada, 2008; Swerdlow et al., 2001). It is tempting to speculate that stress-induced PPI disruptions represent a closer animal model of PTSD-related information-processing deficits than do stress-induced changes in basic startle. This could have important implications for the treatment of PTSD. For example, one might consider multiple treatments to address different symptom/endophenotypic clusters rather than a single drug treatment to treat the “whole” disorder. Of clinical relevance, it would be interesting to see if CRF antagonists given prior to predator exposure in the present model would prevent emergence of the PPI deficits and whether the same treatments would also be effective in normalizing the PPI deficits seen at delayed time points after CRF infusion.

4.6: PPI effects were dissociable from contemporaneous elevations in plasma corticosterone levels

In agreement with a wide array of previous studies, both stressors and CRF caused a marked elevation in plasma corticosterone levels immediately after stress exposure / ICV infusion

(Blanchard et al., 1998; Dunn, 2000; Dunn and File, 1987; Merali et al., 2001). No PPI effects were seen with any stimulus at this time point, suggesting that acute HPA activation was not sufficient to cause a PPI deficit. Although footshock and ferret stress caused identical levels of corticosterone release at this single time point, it is still possible that the corticosterone effects might have differed at other time points. For example, perhaps the duration of acute predator stress-induced corticosterone elevation was significantly longer than that of the footshock, and it is possible that this contributed to the differential development of PPI deficits later on. The highest level of corticosterone elevation was produced acutely by the high dose of CRF; this level was significantly higher than all others. Given that this was also the only treatment that caused the most extended (9d) deficit in PPI, it is conceivable that this higher level of corticosterone release contributed to the unique PPI profile that was produced by this CRF dose. Regardless, contemporaneous corticosterone elevation (as an index of HPA axis activation) was not necessary for the existence of PPI deficits, as evidenced by the fact that at the 24h time point, when PPI deficits were seen with predator stress and CRF, corticosterone levels for all treatments had normalized to control levels. Although acute cortisol has been found to produce a PPI disruption previously (Ingram et al., 2005; Richter et al., 2011), it has been shown that CRF-induced PPI deficits are independent of glucocorticoid release (Groenink et al., 2008), which is consistent with the present results.

4.7: Conclusions

In summary, the delayed-onset and long-lasting nature of predator stress and CRF-induced PPI deficits provides an important working model from which testable hypotheses can be generated regarding prophylactic (Zohar et al., 2009) versus post-hoc treatments for PTSD and other illnesses (like schizophrenia), in which PPI deficits are seen and in which symptom exacerbation occurs after intense stress.

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Figure 1.

Effects of ferret stress on % prepulse inhibition for prepulses that are 3, 6, or 12 dB above background noise level (x-axis). Graphs show data for multiple time points after ferret presentation: **A)** Acute (immediate); **B)** 24h; **C)** 48h; **D)** 9d. Values represent means $\pm$ sem for each group. ** $P < 0.01$, * $P < 0.05$, relative to No Stress group.

Figure 2.

Effects of footshock on % prepulse inhibition for prepulses that are 3, 6, or 12 dB above background noise level (x-axis). Graphs show data for multiple time points after footshock presentation: **A)** Acute (immediate); **B)** 24h; **C)** 48h; **D)** 9d. Values represent means $\pm$ sem for each group.

Figure 3.

Effects of intracerebroventricular infusion of corticotropin-releasing factor (CRF, 3μg/6μl) on **A)** locomotion (cage crossings), and **B)** grooming. Effects on % prepulse inhibition of the same treatments **C)** 15 min, or **D)** 30 min after infusion, at prepulse intensities that are 3, 6, and 12 dB above background (x-axis). All values represent means ± sem for each group.

**P<0.01, *P<0.05, relative to corresponding vehicle values.

Figure 4.

Effects of corticotropin-releasing factor (CRF, 3μg/6μl) on % prepulse inhibition for prepulses that are 3, 6, or 12 dB above background noise level (x-axis). Graphs show data for multiple time points after intracerebroventricular infusion: **A)** Acute (45 min); **B)** 24h; **C)** 48h; **D)** 9d. Values represent means ± sem for each group. *P<0.05, relative to vehicle group.

Figure 5.

Effects of a lower dose of corticotropin-releasing factor (CRF, 0.5 $\mu\text{g}/6\mu\text{l}$) on % prepulse inhibition for prepulses that are 3, 6, or 12 dB above background noise level (x-axis). Graphs show data for multiple time points after intracerebroventricular infusion: **A)** Acute (45 min); **B)** 24h; **C)** 48h; **D)** 9d. Values represent means $\pm$ sem for each group. * $P < 0.05$, relative to vehicle group.

Figure 6.

Effects on baseline startle magnitude at multiple time points after **A)** ferret exposure, **B)** footshock, **C)** high-dose intracerebroventricular corticotropin-releasing factor (CRF, 3 µg/6 µl), **D)** low-dose intracerebroventricular corticotropin-releasing factor (CRF, 0.5 µg/6 µl).

*P<0.05, relative to corresponding vehicle values.

Figure 7.

Effects on plasma corticosterone levels at acute and 24-h time points after **A)** ferret or footshock exposure, and **B)** intracerebroventricular infusion of corticotropin-releasing factor (CRF). Values represent means $\pm$ sem for each group. Doses are in μ g/6 μ l. *** P <0.001, ** P <0.01, * P <0.05, relative to corresponding no-stress/vehicle condition. + P <0.05, compared to the corresponding 'CRF 0.5' group.