

Reversal of Startle Gating Deficits in Transgenic Mice Overexpressing Corticotropin-Releasing Factor by Antipsychotic Drugs

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Chronically elevated levels of corticotropin-releasing factor (CRF) in transgenic mice overexpressing CRF in the brain (CRF-OE) appear to be associated with alterations commonly associated with major depressive disorder, as well as with sensorimotor gating deficits commonly associated with schizophrenia. In the present study, we tested the hypothesis that antipsychotics may be effective in normalizing prepulse inhibition (PPI) of acoustic startle in CRF-OE mice, which display impaired sensorimotor gating compared to wild-type (WT) mice. The typical antipsychotic haloperidol and atypical antipsychotic risperidone improved PPI in the CRF-OE mice, but were ineffective in WT mice. The atypical antipsychotic clozapine did not influence PPI in CRF-OE mice, but reduced gating in WT mice. This effect of clozapine in the CRF-OE mice may thus be regarded as a relative improvement, consistent with the observed effect of haloperidol and risperidone. As expected, the anxiolytic, nonantipsychotic chlordiazepoxide was devoid of any effect. All four compounds dose-dependently reduced the acoustic startle response irrespective of genotype. These results indicate that antipsychotic drugs are effective in improving startle gating deficits in the CRF-OE mice. Hence, the CRF-OE mouse model may represent an animal model for certain aspects of psychotic depression, and could be a valuable tool for research addressing the impact of chronically elevated levels of CRF on information processing.

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INTRODUCTION

Among the various peptide and neurotransmitter systems that have been implicated in the regulation of stress responses, the corticotropin-releasing factor (CRF, Hauger *et al*, 2003; also referred to as CRH) system takes a prominent position (Dunn and Berridge, 1990; Owens and Nemeroff, 1991; Koob *et al*, 1993; Holsboer, 1999; Koob and Heinrichs, 1999). As such, chronically elevated levels of CRF are implicated in stress-related and affective disorders in humans, including major depressive disorder (see Mitchell, 1998; Arborelius *et al*, 1999; Meyer *et al*, 2001). Core symptoms of major depression include decreased mood and loss of interest in otherwise pleasurable events and things (DSM-IV). Depression also disturbs fundamental biological

processes that regulate autonomic function, neuroendocrine regulation, sleep, appetite, and sexual activity (see Meyer *et al*, 2001).

To gain more insight into the relationship between chronically elevated levels of CRF and associated neuroendocrine, autonomic, physiological, and behavioral changes, we have developed a transgenic mouse model of life-long CRF overproduction (CRF-OE) (Dirks *et al*, 2002b; Groenink *et al*, 2002), under control of the Thy-1 promoter which drives constitutive transgene expression in neurons in postnatal and adult brain (eg Morris and Grosveld, 1989; Vidal *et al*, 1990; Moechars *et al*, 1996; Lüthi *et al*, 1997; Wiessner *et al*, 1999). Chronic CRF overproduction in the transgenic mice appears to be associated with chronic stress-like alterations, including increased CRF expression and CRF-immunoreactivity in the hypothalamus, increased heart rate and body temperature, decreased heart rate variability, and altered hypothalamus–pituitary–adrenal (HPA) axis activity and regulation, reflected in increased basal plasma corticosterone concentrations, adrenal gland hypertrophy, and nonsuppression of corticosterone secretion in response to dexamethasone (Dirks *et al*, 2002b; Groenink *et al*, 2002).

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Apart from these physiological alterations, results of a previous study in CRF-OE mice also indicated that chronic hyperactivity of the CRF system is associated with reductions in startle reactivity and prepulse inhibition (PPI) (Dirks *et al*, 2002a). The startle reflex is a fast, involuntary contraction of facial and body muscles accompanied by eyelid closure as well as an arrest of ongoing behaviors, evoked by sudden and intense acoustic stimuli (Koch, 1999). PPI is the reduction of the startle response that occurs if the startle stimulus is preceded 30–500 ms by a distinctive, nonstartling stimulus (prepulse) (Graham, 1975). It is used as an operational measure for early sensorimotor gating (Braff and Geyer, 1990), which is a neural mechanism theorized to maintain mental and behavioral integration by inhibiting irrelevant sensory and cognitive information and motor programs (Dulawa *et al*, 2000). Impaired PPI has been demonstrated in patients with several neuropsychiatric disorders, most notably schizophrenia (Braff *et al*, 1978, 1992; for a recent review, see Braff *et al*, 2001), but not in patients with major depressive disorder without psychosis (W Perry, A Minassian, and D Feifel, personal communication; Ludewig and Ludewig, 2003). Interestingly, acutely administered CRF reduces PPI in rats (Conti *et al*, 2002) and mice (V Risbrough and M Geyer, personal communication), independent of its effects on startle reactivity. These findings indicate that both acute and chronic elevated levels of CRF have similar disruptive effects on PPI.

A distinct subtype of major depression is psychotic or delusional depression (see Schatzberg and Rothschild, 1992). It is generally referred to as major depression with psychotic features, and is characterized by the presence of delusions or hallucinations (typically auditory) during a major depressive episode (DSM-IV). As such, patients with psychotic depression are preferentially treated with antidepressants, mostly tricyclic antidepressants (TCAs), in combination with antipsychotic drugs (see Wheeler Vega *et al*, 2000). Recently, it has been suggested that patients with psychotic depression, who have a perturbed HPA axis, also have great difficulties in distinguishing between relevant and irrelevant stimuli (Belanoff *et al*, 2001). Interestingly, the CRF-OE mice can be characterized by alterations commonly associated with major depression (Dirks *et al*, 2002b; Groenink *et al*, 2002), as well as by sensorimotor gating deficits commonly associated with schizophrenia (Dirks *et al*, 2002a). Therefore, CRF-OE mice might represent an animal model for certain aspects of psychotic depression. To gather further support for this hypothesis, the effects of several antipsychotic drugs on startle reactivity and PPI were assessed in the present study. If indeed the CRF-OE mice can be regarded as representative for psychotic depression, antipsychotic drugs, including haloperidol, clozapine, and risperidone, should reverse the observed startle gating deficits. Nonantipsychotics, such as the anxiolytic chlordiazepoxide, should be devoid of any effect. Furthermore, given its sedative properties, this drug could be used as a control for any antipsychotic-induced sedation.

MATERIALS AND METHODS

Animals

CRF-OE mice were generated as described previously (Dirks *et al*, 2002a, b). Briefly, the CRF transgene was composed of

the complete coding sequence of rat CRF cDNA (0.6 kb fragment; Thompson *et al*, 1987), which was inserted into a 8.2 kb genomic DNA fragment encompassing the murine Thy-1.2 gene, including regulatory regions and polyadenylation signal sequence (Aigner *et al*, 1995). The Thy-1 regulatory sequences drive constitutive transgene expression in postnatal and adult neurons (Morris and Grosveld, 1989; Vidal *et al*, 1990; Moechars *et al*, 1996; Lüthi *et al*, 1997; Wiessner *et al*, 1999). Subsequent breeding at the local breeding facilities (Central Laboratory Animal Institute, Utrecht, The Netherlands) consisted of matings between heterozygous transgenic males (C57BL/6J background) and C57BL/6Jlco females (obtained from Charles River, The Netherlands).

Male transgenic CRF-OE mice (line 2122, >7th generation) were used in these experiments. Littermate wild-type (WT) mice served as controls. Animals were group-housed at constant room temperature ($21 \pm 2^\circ\text{C}$) and relative humidity (50–60%), with EnviroDri[®] (BMI, Helmond, The Netherlands) and a piece of PVC-tubing (diameter 5 cm) as cage-enrichment. Standard rodent food pellets (Special Diet Services Ltd., Witham, Essex, UK) and water were freely available. Mice were maintained on a 12 h light–dark cycle (lights on from 0600 to 1800). All experimental procedures were conducted during the light phase of the cycle. These studies were approved by the ethical committee on animal experiments of the Faculties of Pharmacy, Biology, and Chemistry of Utrecht University, The Netherlands.

Drugs

Haloperidol (0.3, 1.0, and 3.0 mg/kg; Sigma-RBI, Zwijndrecht, The Netherlands), clozapine (1, 3, and 10 mg/kg; Sigma-RBI), and risperidone (0.25, 0.5, and 1.0 mg/kg; Sigma-RBI) were dissolved in a vehicle containing saline and 0.3% Tween[®] 80 (Fluka, Buchs, Switzerland). Chlordiazepoxide (5, 10, and 20 mg/kg, CDP; Pharbita, Zaandam, The Netherlands) was dissolved in saline (0.9% NaCl). All drugs were administered intraperitoneally (i.p.) in a volume of 10 ml/kg, 30 min before the start of the test.

Apparatus

Startle reflexes were measured in four identical startle response systems (SR-LAB, San Diego Instruments, San Diego, CA, USA) consisting of a nonrestrictive Plexiglas cylinder (inner diameter 4 cm, length 13 cm) and grid floor, mounted on a Plexiglas platform and placed in a ventilated, sound-attenuated chamber. Cylinder movements were detected and measured by a piezoelectric element mounted under each cylinder. A dynamic calibration system (San Diego Instruments) was used to ensure comparable startle magnitudes across the four devices. Startle stimuli were presented through a high-frequency speaker located 33 cm above the startle chambers. The background noise was 70 dB. Startle magnitudes were sampled each millisecond (ms) during a period of 65 ms beginning at the onset of the startle stimulus. A startle response is defined as the peak response during this 65 ms period.

Procedure

A modified procedure of that described by Dulawa and Geyer was adopted (Dulawa and Geyer, 2000; Dulawa *et al.*, 2000).

Matching. Three days prior to the test sessions, mice were exposed to 20 acoustic stimuli (100 and 110 dB noise, 50 ms, interstimulus interval (ISI) 30 s). The purpose of this matching procedure was to familiarize the mice to handling, the startle apparatus, and the startle stimulus, and to compose drug groups of mice with equal mean startle magnitudes.

Test sessions. Mice were exposed to five different trial types within a 45-min session. Startle stimuli (110 dB, 50 ms) were presented alone or were preceded by noise prepulses (20 ms) of 4, 8, or 16 dB above background (ie 74, 78, or 86 dB) with a fixed interval (100 ms) between onsets of the prepulse and startle stimuli. Also, no-stimulus trials were included in which only the background noise was presented to assess spontaneous activity during testing. The test session began with a 5-min acclimatization period followed by three consecutive blocks of test trials. Block one consisted of six consecutive startle stimulus-alone trials, while blocks two and three each contained six startle stimulus-alone trials, five startle+prepulse trials per prepulse intensity, and five no-stimulus trials. Trials were presented in a pseudorandom order within a block. Intertrial intervals ranged from 25 to 35 s.

Experimental design. Each drug was tested in a separate group of animals consisting of CRF-OE and littermate WT mice, age 10–23 weeks (haloperidol: WT $n=15$, CRF-OE $n=14$; clozapine: WT $n=16$, CRF-OE $n=15$; risperidone: WT $n=15$, CRF-OE $n=13$; CDP: WT $n=14$, CRF-OE $n=12$). Per drug, mice were tested in a within-subject design, receiving each dose and vehicle once, according to Latin-square treatment protocol. Tests were separated from each other by at least 1 week. Body weight was determined weekly on the day before testing.

Data Analysis

Startle magnitude is expressed as arbitrary units in all the procedures. Results were evaluated for each drug separately.

Averaged block two and three startle values, which were used for the calculation of PPI, were analyzed by a repeated measures ANOVA with dose as a within-subject factor (four levels) and genotype as a between-subject factor (two levels) to assess startle reactivity.

Percent PPI was calculated as the mean startle magnitude to startle stimulus-alone, minus the mean startle magnitude to startle+prepulse trials, all divided by the mean startle stimulus-alone trials, and multiplied by 100. Block one startle magnitude values were not used in the calculation of PPI because they reflect the initial rapid habituation of startle and are highly variable (see Dulawa *et al.*, 2000). Results were analyzed by means of repeated measures ANOVA with dose (four levels) and prepulse intensity (three levels) as within-subject factors and genotype (two levels) as a between-subject factor. In the case of significant

dose \times prepulse intensity interaction, data were reanalyzed by means of repeated measures ANOVAs with dose as a within-subject factor and genotype as a between-subject factor for each prepulse intensity separately. In the case of significant dose \times genotype interaction, data were reanalyzed by means of repeated measures ANOVAs with dose and prepulse intensity as within-subject factors for each genotype separately. *Post hoc* tests consisted of independent *t*-tests with Bonferroni correction of α .

Body weight across experiments was analyzed by means of repeated measures ANOVA with week (four levels) as a within-subject factor and genotype (two levels) as a between-subject factor.

If appropriate, the Greenhouse–Geisser correction factor (ϵ) was used in repeated measures ANOVAs to adjust the degrees of freedom (Vasey and Thayer, 1987). The level of significance was set at $p < 0.05$.

RESULTS

Acoustic Startle

Figure 1 shows the effects of haloperidol (0, 0.3, 1.0, and 3.0 mg/kg), clozapine (0, 1, 3, and 10 mg/kg), risperidone (0, 0.25, 0.5, and 1.0 mg/kg), and chlordiazepoxide (0, 5, 10, and 20 mg/kg) on the averaged startle response in blocks two and three. Within each drug cohort, startle magnitudes of vehicle-treated WT and CRF-OE mice were not significantly different (haloperidol $F(1,27)=0.04$, NS; clozapine $F(1,29)=1.11$, NS; risperidone $F(1,26)=0.21$, NS; chlordiazepoxide $F(1,24)=0.66$, NS).

All four compounds decreased startle reactivity irrespective of genotype (haloperidol: dose $F(3,81)=4.78$, $p < 0.05$, $\epsilon=0.63$, genotype $F(1,27)=0.12$, NS, dose \times genotype $F(3,81)=0.44$, NS, $\epsilon=0.63$; clozapine: dose $F(3,87)=37.98$, $p < 0.001$, $\epsilon=0.67$, genotype $F(1,29)=1.68$, NS, dose \times genotype $F(3,87)=0.52$, NS, $\epsilon=0.67$; risperidone: dose $F(3,78)=44.49$, $p < 0.001$, genotype $F(1,26)=0.32$, NS, dose \times genotype $F(3,78)=0.13$, NS; chlordiazepoxide: dose $F(3,72)=3.67$, $p < 0.05$, $\epsilon=0.77$, genotype $F(1,24)=2.09$, NS, dose \times genotype $F(3,72)=2.04$, NS, $\epsilon=0.77$).

Prepulse Inhibition

Figure 2 shows the effects of haloperidol (0, 0.3, 1.0, and 3.0 mg/kg), clozapine (0, 1, 3, and 10 mg/kg), risperidone (0, 0.25, 0.5, and 1.0 mg/kg), and chlordiazepoxide (0, 5, 10, and 20 mg/kg) on percent PPI scores. Within each drug cohort, a significant main effect for genotype was observed in vehicle-treated animals (haloperidol $F(1,27)=8.03$, $p < 0.01$; clozapine $F(1,29)=18.73$, $p < 0.001$; risperidone $F(1,26)=7.58$, $p < 0.05$; chlordiazepoxide $F(1,24)=12.61$, $p < 0.005$). Furthermore, in the haloperidol and risperidone cohorts, there was also a significant genotype \times prepulse intensity interaction (haloperidol $F(2,54)=5.89$, $p < 0.01$; clozapine $F(2,58)=0.70$, NS, $\epsilon=0.81$; risperidone $F(2,52)=8.87$, $p < 0.001$; chlordiazepoxide $F(2,48)=0.90$, NS, $\epsilon=0.68$).

The percent PPI was monotonically related to the intensity of the prepulse in all cohorts (Figure 2; haloperidol: $F(2,54)=281.31$, $p < 0.001$; clozapine: $F(2,58)=140.28$, $p < 0.001$; risperidone: $F(2,52)=255.93$, $p < 0.001$, $\epsilon=0.64$;

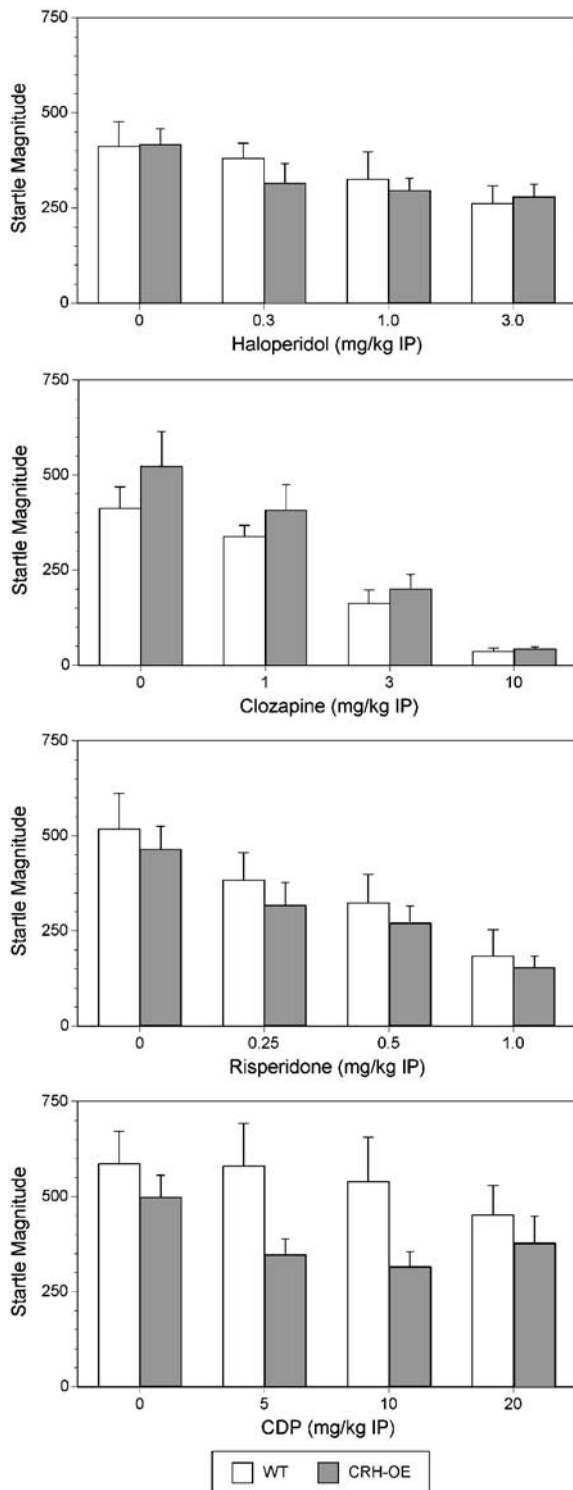


Figure 1 Effects of haloperidol, clozapine, risperidone, and chlordiazepoxide on the acoustic startle response in WT (white bars) and CRF-OE (gray bars) mice. Haloperidol: WT $n = 15$, CRF-OE $n = 14$; clozapine: WT $n = 16$, CRF-OE $n = 15$; risperidone: WT $n = 15$, CRF-OE $n = 13$; CDP: WT $n = 14$, CRF-OE $n = 12$.

chlordiazepoxide: $F(2,48) = 305.96$, $p < 0.001$, $\epsilon = 0.75$). Furthermore, CRF-OE mice displayed impaired levels of PPI as a function of the prepulse intensity, reflected in (nearly) significant main effects of genotype (haloperidol:

$F(1,27) = 13.60$, $p < 0.005$; clozapine: $F(1,29) = 5.34$, $p < 0.05$; risperidone: $F(1,26) = 3.25$, $p = 0.083$; chlordiazepoxide: $F(1,24) = 20.37$, $p < 0.001$), and significant prepulse intensity \times genotype interactions (haloperidol: $F(2,54) = 14.21$, $p < 0.001$; clozapine: $F(2,58) = 9.75$, $p < 0.001$; risperidone: $F(2,52) = 7.84$, $p < 0.01$, $\epsilon = 0.64$; chlordiazepoxide: $F(2,48) = 4.24$, $p < 0.05$, $\epsilon = 0.75$).

As shown in Figure 2, haloperidol increased PPI dose-dependently ($F(3,81) = 5.64$, $p < 0.005$), regardless of genotype (dose \times genotype: $F(3,81) = 2.16$, NS), but dependent on prepulse intensity (dose \times prepulse intensity: $F(6,162) = 2.82$, $p < 0.05$, $\epsilon = 0.72$; dose \times prepulse intensity \times genotype: $F(6,162) = 1.40$, NS, $\epsilon = 0.72$). Lower order ANOVAs on each prepulse intensity separately revealed a significant dose \times genotype interaction only at the 8-dB prepulse intensity. *Post hoc* independent *t*-tests revealed that the differences in PPI between CRF-OE and WT mice were no longer evident after 1.0 and 3.0 mg/kg haloperidol.

Clozapine affected PPI dose-dependently and genotype-dependently (Figure 2; dose: $F(3,87) = 8.12$, $p < 0.001$; dose \times genotype: $F(3,87) = 11.74$, $p < 0.001$; dose \times prepulse intensity: $F(6,174) = 5.73$, $p < 0.001$, $\epsilon = 0.65$; dose \times prepulse intensity \times genotype: $F(6,174) = 1.34$, NS, $\epsilon = 0.65$). When analyzing each prepulse intensity separately, as permitted by the significant dose \times prepulse intensity interaction, repeated measures ANOVAs revealed significant dose \times genotype interactions at all prepulse intensities. Accompanying *post hoc* independent *t*-tests revealed that the differences in PPI between CRF-OE and WT mice were no longer evident after 3 and 10 mg/kg clozapine. When analyzing each genotype separately, as allowed by the significant dose \times genotype interaction, analyses reveal that the 10-mg/kg clozapine dose induced a significant reduction of PPI in the WT mice at the 8-dB prepulse intensity, while 3 and 10 mg/kg did so at 16 dB. In CRF-OE mice, clozapine did not affect PPI differentially at different prepulse intensities, reflected by a nonsignificant dose \times prepulse interaction.

After risperidone treatment, PPI was also changed dose-dependently and genotype-dependently (Figure 2; dose: $F(3,78) = 1.50$, NS; dose \times genotype: $F(3,78) = 4.31$, $p < 0.01$; dose \times prepulse intensity: $F(6,156) = 3.46$, $p < 0.005$; dose \times prepulse intensity \times genotype: $F(6,156) = 2.08$, $p = 0.058$). When analyzing each prepulse intensity separately, as permitted by the significant dose \times prepulse intensity interaction, repeated measures ANOVAs revealed significant main effects of genotype and significant dose \times genotype interactions at prepulse intensities of 4 and 8 dB with CRF-OE mice showing significantly less PPI than WT mice after vehicle treatment. This difference between genotypes disappeared after risperidone treatment. When analyzing the genotypes separately, risperidone improved PPI in the CRF-OE mice, particularly at 0.25 mg/kg after 4 dB prepulses and at 1.0 mg/kg after 8 dB prepulses. In WT mice, risperidone did not affect PPI differentially at different prepulse intensities, reflected by a nonsignificant dose \times prepulse interaction.

As expected, chlordiazepoxide had no effect on PPI (Figure 2; dose: $F(3,72) = 0.41$, NS; dose \times genotype: $F(3,72) = 0.63$, NS; dose \times prepulse intensity: $F(6,144) = 1.64$, NS, $\epsilon = 0.48$; dose \times prepulse intensity \times genotype: $F(6,144) = 0.87$, NS, $\epsilon = 0.48$).

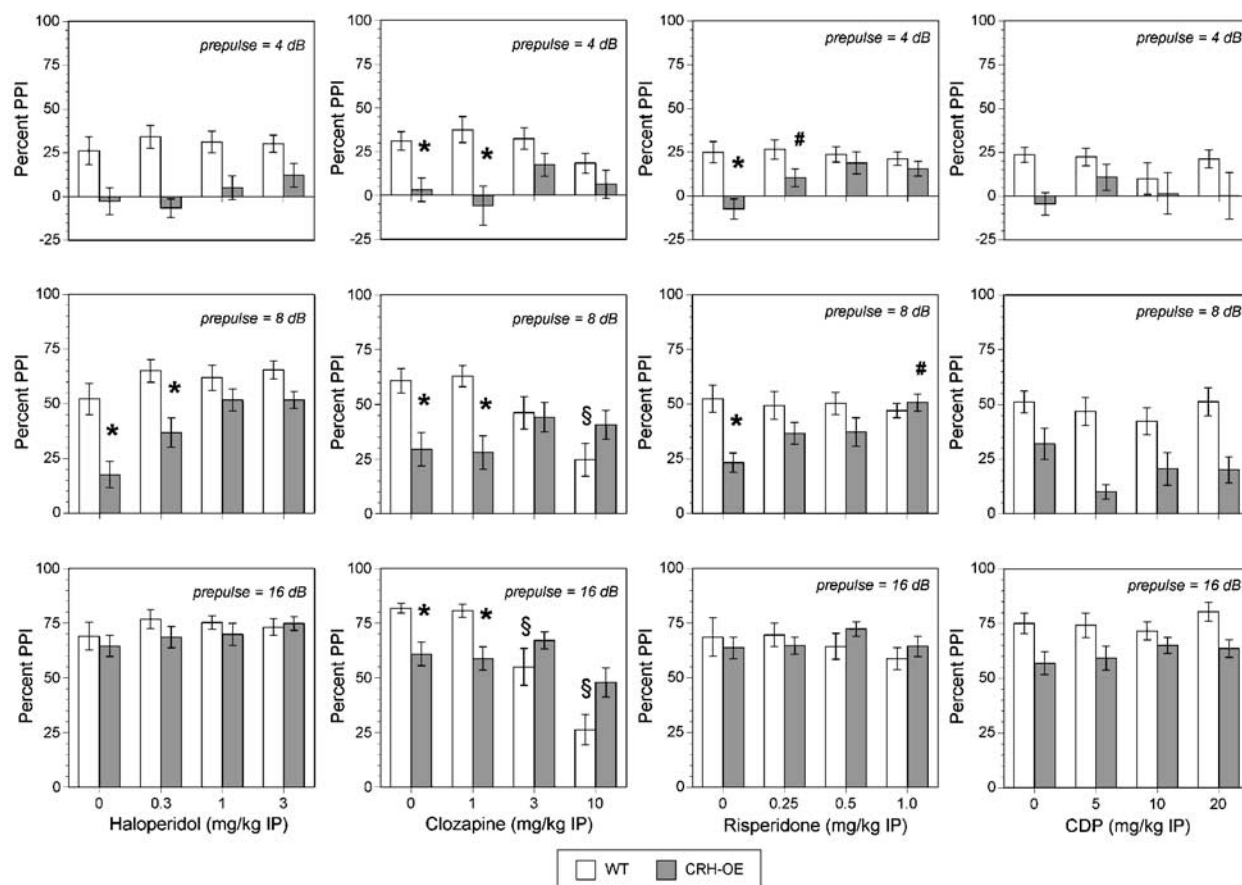


Figure 2 Effects of haloperidol, clozapine, risperidone, and chlordiazepoxide on prepulse inhibition of the acoustic startle response in WT (white bars) and CRF-OE (gray bars) mice. The main effect of genotype was significant in each of the four cohorts of mice tested. Haloperidol: WT $n = 15$, CRF-OE $n = 14$; clozapine: WT $n = 16$, CRF-OE $n = 15$; risperidone: WT $n = 15$, CRF-OE $n = 13$; CDP: WT $n = 14$, CRF-OE $n = 12$. * $p < 0.05$ vs same-dose-treated WT mice, § $p < 0.05$ vs vehicle-treated WT mice, # $p < 0.05$ vs vehicle-treated CRF-OE mice.

Body Weight

At the start of the experiments, CRF-OE mice had significantly lower body weights than WT (WT: $n = 60$, 29.0 ± 0.4 g; CRF-OE: $n = 54$, 25.5 ± 0.5 g; $t = 5.97$, $df = 112$, $p < 0.001$). These differences in body weight between genotypes remained present throughout all procedures (week: $F(3,336) = 74.06$, $p < 0.001$; genotype: $F(1,112) = 32.18$, $p < 0.001$; week \times genotype: $F(3,336) = 1.83$, NS; $\epsilon = 0.73$). The same holds true for each drug cohort separately.

DISCUSSION

The main objective of this study was to test the hypothesis that antipsychotics may be effective in improving PPI in the CRF-OE mice, which displayed impaired sensorimotor gating when compared to WT mice in each of the four cohorts examined. Indeed, both the typical antipsychotic haloperidol and atypical antipsychotic risperidone improved PPI in the CRF-OE mice, while they were ineffective in WT mice. The atypical antipsychotic clozapine did not have an effect on PPI in the CRF-OE mice, although it reduced gating in WT mice. This effect of clozapine in the CRF-OE mice may thus be regarded as a relative improve-

ment, consistent with the observed effect of haloperidol and risperidone. As expected, the nonantipsychotic and anxiolytic chlordiazepoxide were devoid of any effect. These results indicate that antipsychotic drugs are effective in reversing the startle gating deficits in the CRF-OE mice.

In the four independent cohorts of mice tested here, we replicated previous results showing decreased startle plasticity as evidenced by impaired PPI in the CRF-OE mice, although the concomitant decrease in startle reactivity observed previously was not confirmed in the present experiments (Dirks *et al.*, 2002a), possibly due to the confounding effects of injection stress. As CRF-OE mice overproduce CRF throughout postnatal development, numerous neurochemical and developmental changes may have occurred to compensate for the increased levels of this neuropeptide. Thus, it cannot be ruled out that the observed phenotype is not the direct consequence of chronically CRF excess, but rather the result of compensatory adaptations in other neurotransmitter systems involved in startle reactivity and startle plasticity. Nevertheless, it was recently reported that acutely administered CRF reduces PPI in two strains of rats, independent of its effects on acoustic startle amplitude (Conti *et al.*, 2002). This observation, coupled with preliminary observations of similar effects of CRF on PPI in two strains of mice (V. Risbrough and M. Geyer, personal

communication), indicates that both acute administrations and chronic overexpression of CRF have similar disruptive effects on PPI. Another possible concern is that the lower body weight in CRF-OE mice might have contributed to the observed difference in startle reactivity. This possibility appears to be unlikely because there was no significant correlation between acoustic startle magnitude and body weight (data not shown). Another potential concern when testing PPI in mice involves hearing deficits. C57BL/6 mice hear normally as young adults (1–2 months of age), but exhibit high-frequency hearing loss (>20 kHz) by 5–6 months, accompanied by increased behavioral salience of low and middle frequencies (eg Parham and Willott, 1988; Willott *et al*, 1994; Carlson and Willott, 1996). In this case, however, the WT and CRF-OE mice on a C57BL/6J background were well below the critical age, and we used noise with a broad sound frequency spectrum for both prepulse and startle stimuli, thus avoiding stimuli with just one frequency. Furthermore, if the reduced PPI in the CRF-OE mice were due to hearing deficits, such hearing deficits would have to be reversible by acute administrations of antipsychotic drugs, as seen in the present studies. Hence, we think that it is highly unlikely that high-frequency hearing loss contributed to the observed effects.

In this study, the effects of haloperidol (typical antipsychotic, dopamine D_2 receptor antagonist), clozapine (atypical antipsychotic, $D_1/D_2/D_3/D_4/5$ -HT $_2/\alpha_1$ /muscarinic receptor antagonist), and risperidone (atypical antipsychotic, $D_2/5$ -HT $_2/\alpha_1/\alpha_2$ receptor antagonist) on startle reactivity and PPI were investigated in CRF-OE and WT mice. The three compounds dose-dependently reduced the acoustic startle response irrespective of genotype. These findings correspond well with reported effects of these antipsychotics in mice. Although it has been reported that haloperidol increases startle reactivity over a wide dose range in mice (McCaughan Jr *et al*, 1997), others report a decrease in startle response after haloperidol treatment in mice, as is commonly seen in rats (Mansbach *et al*, 1988; Ouagazzal *et al*, 2001). Also, clozapine decreases startle response in mice of different background strains, including C57BL/6J (Olivier *et al*, 2001; Ouagazzal *et al*, 2001). In C57BL/6J mice, risperidone had either no effect on startle amplitude (McCaughan Jr *et al*, 1997; Olivier *et al*, 2001), or reduced startle reactivity (Ouagazzal *et al*, 2001). In our study, at the two highest doses of clozapine, mice showed clear signs of sedation, which is a known side effect of this drug (see Miller, 2000) that could explain the marked reduction observed in startle reactivity. In concordance, the benzodiazepine chlordiazepoxide reduced startle reactivity most likely due to its sedative properties, although this effect was not robust. Furthermore, catalepsy, which is a good predictor of extrapyramidal side effects of antipsychotic drugs (see Hoffman and Donovan, 1995), was observed in most but not all mice of both genotypes after 0.5 and 1.0 mg/kg risperidone. Unexpectedly, although haloperidol reduced startle reactivity as did risperidone, it did not induce obvious catalepsy. Risperidone- and haloperidol-induced catalepsy have often been described in rodents (eg Kanés *et al*, 1993; Hoffman and Donovan, 1995; Rigdon *et al*, 1996; Wadenberg *et al*, 2001). For risperidone, the ED $_{50}$ value was 1.8 mg/kg PO (in CD-1 mice) and for

haloperidol it was 3.8 mg/kg PO (in C57BL/6J mice) (see Kanés *et al*, 1993; Rigdon *et al*, 1996). Hence, the absence of haloperidol-induced catalepsy in the present study could be related to the dose used (ie ranging from 0.3 to 3.0 mg/kg i.p.).

Both haloperidol and risperidone reversed the disrupted PPI in the CRF-OE mice, while being ineffective in WT mice. These results in the WT mice do not correspond with previous results. In WT C57BL/6J mice, haloperidol has been suggested to facilitate PPI (McCaughan Jr *et al*, 1997), although in another study haloperidol was only effective at the highest prepulse intensity tested (Ouagazzal *et al*, 2001). Also, the atypical antipsychotic risperidone appears to produce dose-dependent improvements in PPI (McCaughan Jr *et al*, 1997; Ouagazzal *et al*, 2001), although this finding is not unequivocal (Olivier *et al*, 2001). Differences between our results in WT mice and those reported in the literature may be due to substrain differences in drug responses and/or differences in stimulus parameters, PPI parameters, and startle measurement methods. The results in the CRF-OE mice, however, are in agreement with previously reported effects of these compounds on PPI deficits in rats. As recently reviewed by Geyer *et al* (2001), pharmacological manipulations with dopamine receptor agonists, 5-HT $_2$ receptor agonists, or NMDA receptor antagonists, or developmental manipulations including isolation rearing, produce robust deficits in PPI in rats, which can be reversed by haloperidol, clozapine, and/or risperidone, depending upon the particular PPI disruption model used. In rats, these compounds generally have minimal effects on PPI by themselves, although some increases or decreases have been reported that appear to depend on strain, startle parameters, and doses used (see Geyer *et al*, 2001).

Unexpectedly, clozapine induced a severe reduction of PPI in WT mice, although it was devoid of any effect in the CRF-OE mice. When analyzing absolute startle values, instead of percentage PPI scores, after 3 and 10 mg/kg clozapine, as allowed by a significant dose \times prepulse intensity interaction in an overall repeated measures ANOVA on absolute startle values (statistics not shown), there is still a significant effect of prepulse intensity irrespective of genotype (statistics not shown), indicating that startle magnitudes are reduced even further, despite startle values of around 50 in the stimulus-alone trials. It is therefore unlikely that the reduction in percent PPI as observed in the WT mice, is derived from a floor effect. Nonetheless, given the strongly reduced startle reactivity after clozapine treatment, the PPI results are difficult to interpret in any definitive way. Clozapine usually potentiates PPI in mice in the same dose range used in this study (McCaughan Jr *et al*, 1997; Olivier *et al*, 2001; Ouagazzal *et al*, 2001). In these studies, saline-treated mice of the C57BL/6J strain showed low levels of PPI, ranging from approximately 10 to 40% (McCaughan Jr *et al*, 1997; Olivier *et al*, 2001; Ouagazzal *et al*, 2001), in concordance with previously reported PPI values for this strain (Paylor and Crawley, 1997). However, in the present and previous study in CRF-OE mice (Dirks *et al*, 2002a), the levels of PPI are substantially higher in the WT C57BL/6J mice dependent on the intensity of the prepulse. These findings suggest that, at least in C57BL/6J mice, the effects of clozapine may depend

on the initial levels of PPI. Thus, the differences in basal PPI levels between present and previous studies may account for the unusual PPI-impairing effect of clozapine observed in the WT C57BL/6J mice. In the CRF-OE mice, clozapine did not induce an impairment of PPI as seen in the WT mice. Given the absence of such clozapine-induced reduction of PPI in the CRF-OE mice, and given that haloperidol and risperidone improved PPI in CRF-OE mice while being ineffective in WT mice, this effect of clozapine in the CRF-OE mice may tentatively be regarded as a relative improvement, and is therefore consistent with the observed effects of haloperidol and risperidone.

We and others have shown repeatedly that sensorimotor gating processes are not generally linked to startle reactivity *per se* and are independent of the magnitude of the startle response in mice (eg Paylor and Crawley, 1997; Ouagazzal et al, 2001; Dirks et al, 2001). The present results are in concordance with these reports. For example, risperidone improved PPI only in CRF-OE mice, while it decreased startle reactivity in both genotypes. It should be noted that it does not always follow that effects of startle can never affect PPI scores, and should not be considered as a potential confound of PPI results (see Swerdlow et al, 2000).

The neurodevelopmental hypothesis of schizophrenia suggests that an abnormal development of the brain connectivity resulting from a genetic predisposition in combination with pre- or early postnatal environmental factors, could be one of the mechanisms implicated in the genesis of schizophrenia (Ellenbroek and Cools, 1998; Lipska and Weinberger, 2000). In the CRF-OE mice, the onset of transgene expression, under control of the Thy-1 promoter, occurs shortly after birth reaching maximum levels by postnatal day 14, which are retained throughout adult life (Lüthi et al, 1997). Hence, the early postnatal onset of CRF overexpression and associated consequences for neuroendocrine, neurochemical, autonomic, and developmental processes, could be regarded comparable to other developmental manipulations such as maternal deprivation, neonatal hippocampus lesioning, and isolation rearing. All these manipulations result in sensorimotor gating deficits in adult life (Geyer et al, 1993; Ellenbroek et al, 1998; Lipska and Weinberger, 2000), which can be reversed by both typical and atypical antipsychotic drugs (Ellenbroek et al, 1998; Geyer et al, 2001; Le Pen and Moreau, 2002). The results of the present study suggest that the CRF-OE mouse model might be an additional animal model to study the long-term effects of early postnatal homeostatic disturbances on sensorimotor gating.

In conclusion, the present study shows that antipsychotic drugs (haloperidol, risperidone, and clozapine) are effective in reversing startle gating deficits in the CRF-OE mice, independent of their effects on startle reactivity *per se*. Impaired PPI has been demonstrated in patients with several neuropsychiatric disorders, most notably schizophrenia (Braff et al, 1978, 1992); for a recent review, see Braff et al (2001), but not in patients with major depressive disorder without psychosis (W Perry, A Minassian, and D Feifel, personal communication; Ludewig and Ludewig, 2003). However, the effects of antipsychotic treatment on PPI in schizophrenic patients are not consistent, with some studies showing normalization of PPI and others not (see

Braff et al, 2001; Kumari et al, 2002; Kumari and Sharma, 2002). Psychotic depression, a distinct subtype of major depression, shares many features with schizophrenia, with psychosis being the most prominent (see Schatzberg and Rothschild, 1992). The disorder is generally referred to as major depression with psychotic features, and is characterized by the presence of delusions or auditory hallucinations, and by, among others, excessive HPA axis activity, marked nonsuppression of cortisol secretion after dexamethasone, cognitive and neurophysiological changes, a good response to treatment with an antidepressant plus an antipsychotic, and a joint disturbance of dopaminergic and serotonergic systems (see Anton, 1987; Schatzberg and Rothschild, 1992; Schatzberg et al, 2000; Wheeler Vega et al, 2000; Belanoff et al, 2001). However, to our knowledge, published data on PPI values in patients with psychotic depression are not available. Interestingly, the CRF-OE mice can be characterized by alterations commonly associated with major depression (Dirks et al, 2002b; Groenink et al, 2002) as well as sensorimotor gating deficits commonly associated with schizophrenia or psychosis (Dirks et al, 2002a), which can be reversed by antipsychotic drugs as shown in the present study. Therefore, the CRF-OE mouse model may represent an animal model for certain aspects of psychotic depression, and could be a valuable tool for research addressing the impact of chronically elevated levels of CRF on information processing.

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