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ORIGINAL INVESTIGATION

CRF1 receptor antagonists do not reverse pharmacological disruption of prepulse inhibition in rodents

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Abstract
Rationale As enhanced corticotropin-releasing factor (CRF) transmission is associated with induction of sensorimotor gating deficits, CRF_1 receptor antagonists may reverse disrupted prepulse inhibition (PPI), an operational measure of sensorimotor gating.
Objectives To determine the effects of CRF_1 receptor antagonists in pharmacological models of disrupted PPI and to determine if long-term elevated central CRF levels alter sensitivity towards PPI disrupting drugs.
Methods CP154,526 (10–40 mg/kg), SSR125543 (3–30 mg/kg) and DMP695 (40 mg/kg) were tested on PPI disruption provoked by D-amphetamine (2.5, 3 mg/kg), ketamine (5, 30 mg/kg) and MK801 (0.2, 0.5 mg/kg) in Wistar rats, C57Bl/6J and CD1 mice, and on spontaneously low PPI in Iffa Credo rats and DBA/2J mice. PPI-disrupting effects of D-amphetamine (2.5–5 mg/kg) and MK801 (0.3–1 mg/kg) were examined in CRF-overexpressing (CRFtg) mice, which display PPI deficits. Finally, we determined the influence of CP154,526 on D-amphetamine-induced dopamine outflow in nucleus accumbens and prefrontal cortex of CRFtg mice using in vivo microdialysis.
Results No CRF_1–antagonists improved PPI deficits in any test. CRFtg mice showed blunted PPI disruption in response to MK801, but not D-amphetamine. Further, D-amphetamine-induced dopamine release was *less* pronounced in CRFtg versus wild-type mice, a response normalized by pretreatment with CP154,526.
Conclusion The inability of CRF_1 receptor antagonists to block pharmacological disruption of sensorimotor gating suggests that the involvement of CRF1 receptors in the modulation of dopaminergic and glutamatergic neurotransmission relevant for sensory gating is limited. Furthermore, the alterations observed in CRFtg mice support the notion that long-term elevated central CRF levels induce changes in these neurotransmitter systems.

Keywords Sensorimotor gating · CP154,526 · DMP 695 · SSR125543 · Dopamine · Microdialysis · Dizocilpine · Corticotropin-releasing hormone

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Introduction

Several lines of evidence point to dysfunction of corticotropin-releasing factor (CRF) systems in psychosis. A decrease in CRF binding protein was found in the amygdala of male schizophrenic and bipolar patients (Herringa et al. 2006), and positive treatment response to the antipsychotic quetiapine was associated with decreased levels of CRF in cerebrospinal fluid of schizophrenia patients (Nikisch et al. 2012). Also, posttraumatic stress disorder (PTSD) patients with secondary psychotic features have significantly higher CRF levels in cerebrospinal fluid than non-psychotic PTSD and control subjects (Sautter et al. 2003).

Furthermore, animal studies have provided evidence that CRF_1 receptors are involved in stress-triggered psychostimulant "seeking" and activation of mesolimbic dopaminergic projections, processes related to onset of psychosis (Haass-Koffler and Bartlett 2012).

In addition to psychosis, CRF is implicated in disrupting prepulse inhibition (PPI) of the acoustic startle response, an operational measure of sensorimotor gating (Graham 1975; Braff et al. 1978; Swerdlow et al. 2008). The startle response is a fast, involuntary contraction of bodily muscles, evoked by a sudden and intense acoustic stimulus. When a startling stimulus is preceded by a prepulse, a weak, non-startling sensory stimulus with a 30- to 500-ms lead time, the magnitude of the startle response is reduced; a process known as PPI (Braff et al. 2001). Levels of PPI are thought to reflect the extent to which the prepulse activates preattentive mechanisms which inhibit pulse processing, to buffer sensory processing and prevent sensory flooding (Dulawa and Geyer. 2000). Deficient PPI has been observed in several psychiatric disorders, including schizophrenia, obsessive–compulsive disorder, and Gilles de la Tourette syndrome (for a recent review, see Kohl et al. 2013; Millan et al. 2012). In schizophrenia patients PPI deficits have been associated with cognitive fragmentation, thought disorder and both positive and negative symptoms (Braff et al. 1999; Millan et al. 2012).

In rodents, disruption of PPI is used as a tool for screening of antipsychotic drug properties (Braff et al. 2001; Swerdlow et al. 2008). PPI deficits are induced using (indirect) dopamine receptor agonists (Ralph et al. 2001) or *N*-methyl-D-aspartate (NMDA) receptor antagonists (Yee et al. 2004), modelling schizophrenia-related functional alterations in dopamine and glutamate associated neurotransmission (Meyer and Feldon 2009). While the dopamine agonist-based models are particularly sensitive to typical antipsychotics, PPI deficits provoked by NMDA antagonists appear more sensitive to atypical antipsychotics (Geyer et al. 2001). Thus, pharmacological disruption of PPI is a well-characterized model of impaired sensory gating in schizophrenia, an important dimension of this complex disorder. And although sensory gating is most closely linked to the emergence of psychosis and other positive symptoms, it is also related to cognitive mechanisms controlling attention.

PPI can also be disrupted by exposure to stressors (reviewed by Douma et al. 2011), long-term CRF overexpression, and acute infusion of CRF into the brains of mice and rats (Dirks et al. 2002b; Risbrough et al. 2004; Conti 2005). The PPI deficits induced by long-term CRF overexpression are normalized by CRF_1 receptor antagonists (Groenink et al. 2008). In addition, CRF-induced PPI deficits can be reversed by typical and atypical antipsychotics (Dirks et al. 2003; Conti 2005). Neuroanatomically, interactions between CRF and dopamine that are relevant for PPI can be expected in the nucleus accumbens (NAc) and medial prefrontal cortex (mPFC). First, CRF_1 receptors are located on dopaminergic neurons in the ventral tegmental area (Van Pett et al. 2000), a major source of dopaminergic input to NAc and mPFC. Second, mPFC and NAc also contain CRF_1 receptors (Van Pett et al. 2000) and are important neurochemical substrates in the dopaminergic regulation of PPI (Swerdlow et al. 2001). Third, CRF has been reported to increase dopaminergic activity in the ventral tegmental area (Wanat et al. 2008), NAc (Kalivas et al. 1987; Pan et al. 1995), and PFC (Dunn and Berridge 1987; Lavicky and Dunn 1993), although decreases have also been reported in the PFC (Kalivas et al. 1987; Izzo et al. 2005). In addition to dopamine, CRF is reported to affect glutamatergic signalling in brain circuits that regulate PPI (Hahn et al. 2009; Wise and Morales 2010; George et al. 2012; Ross and Peselow 2012).

Considering the putative role of CRF in psychosis, and its PPI disruptive effects in rodents, here we studied whether blockade of CRF_1 receptors would improve sensorimotor gating in PPI-disruption models known to be sensitive to acute antipsychotic treatment. We evaluated the effects of CRF_1 receptor antagonists on D-amphetamine-, ketamine-, and MK801-induced PPI disruption, and also on spontaneously low PPI in DBA/2J mice (Olivier et al. 2001) and Iffa Credo Wistar rats (Depoortere et al. 1997). As non-peptidergic CRF_1 receptor antagonists differ in their mode of interactions at CRF_1 receptors — which may possess multiple binding sites and/or various isoforms (Gilligan et al. 2000) — and species differences have been reported with respect to anxiolytic effects of CRF_1 antagonists (Steckler and Holsboer 1999), we tested the effects of three CRF_1 receptor antagonists, CP154,526, DMP695 and SSR125543, in different (sub)strains of Wistar rats and mice (C57Bl/6J, CD1 and DBA/2J mice) across two different labs. All three CRF_1 receptor antagonists have comparable affinity for CRF_1 receptors (2–3 nM) over CRF_2 receptors (>10,000) in vitro, good oral bioavailability and readily cross the blood–brain barrier (reviewed by Zorrilla and Koob 2004). For CP154,526, oral bioavailability following 30 μmol/kg (10.9 mg/kg) is 27 % in the rat, with maximal plasma concentrations reached 30 min, and maximal brain concentrations reached 1 h post-dosing. Brain concentrations remain stable until 2 h posttreatment, without significant differences between brain areas. CP154,526 has a high brain/plasma ratio of 2.5 (Keller et al. 2002). The oral bioavailability of SSR125543 following 10 mg/kg acute is 53 % in the rat, with a maximal plasma concentration reached 2 h post-dosing. Brain/plasma SSR125543 area under the curve (AUC) ratio is 0.1 (G. Griebel, personal communication). SSR125543 displaced radio ligand binding to the CRF_1 receptor in the brain with an ID50 of 6.5 mg/kg PO (Gully et al. 2002). Available information on DMP695 is more limited; administration of DPM695 (1 mg/kg, iv and po) to dogs generated high plasma levels of moderate duration and good oral bioavailability (59 %) (Gilligan et al.

2000). Further information regarding the biological activity of these CRF_1 receptor antagonists was reviewed by Zorrilla and Koob (2004). Last, to explain the behavioural effects within a mechanistic framework, we performed an in vivo microdialysis study to determine the effect of CP154,526 on D-amphetamine-induced dopamine overflow in NAc and mPFC of transgenic mice overexpressing central CRF from day 4 after birth (CRFtg; Dirks et al. 2002a) and determined whether chronically elevated central CRF levels alter sensitivity to D-amphetamine and MK801 in PPI.

Materials and methods

See Table 1 for a schematic overview detailing information on animals, drugs and experimental procedures used.

Animals

Each drug was tested in a separate cohort of male animals, using a between subjects study design (for details, see Table 1).

At Utrecht University, animals were group-housed (groups of 4) in bedded plastic cages (Makrolon type 2L), enriched with a piece of PVC-tubing and paper tissue, at constant room temperature (21±2 °C) and relative humidity (50–60 %), on a 12:12 light/dark cycle (lights on: 06:00–18:00 hours). For experiments 5 and 6, male littermates were housed together after weaning, resulting in mixed groups (3–6 per cage) of wild-type and CRFtg mice. Standard rodent food pellets (Special Diet Services, Witham, Essex, United Kingdom) and water were available ad libitum.

At Sanofi, animals were maintained under standard laboratory conditions (21±1 °C, 12 h light–dark cycle, lights on at 7AM) with food and water available ad libitum. Rats were housed in groups of six in a colony room.

Table 1 Overview of animals, drugs and experimental procedures used

Study	Laboratory	Animals[a]			Induction				CRF_1 receptor antagonist			
		Species	Strain	Weight/age	Drug	Dose[b] (mg/kg)	Route	i.t.i.	Drug	Dose[c] (mg/kg)	Route	i.t.i.
Dopamine models												
1.1	UU	Rat	Wistar Wi Charles River, NL	250–350 g	D-Amphetamine sulphate	2.5	IP	10 min	SSR125543	30	IP	30 min
1.2	UU	Mouse	C57Bl/6J Charles River, NL	8–16 weeks	D-Amphetamine sulphate	3	IP	10 min	CP154,526	40	IP	30 min
Glutamate NMDA models												
2.1	Sanofi	Mouse	CD1 Iffa Credo, France	30–35 g	MK801 hydrogen maleate	0.2	IP	60 min	SSR125543	10–30	IP	60 min
2.2	UU	Mouse	C57Bl/6J Charles River, NL	8–16 weeks	MK801 hydrogen maleate	0.5	IP	acute	DMP695	40	IP	30 min
3.1	UU	Rat	Wistar Wu Harlan, Horst, NL	250–350 g	Ketamine hydrochloride	5	SC	acute	DMP695	40	IP	30 min
3.2	UU	Mouse	C57Bl/6J Charles River, NL	8–16 weeks	Ketamine hydrochloride	30	IP	acute	CP154,526	40	IP	30 min
Low PPI animal strains												
4.1	Sanofi	Rat	Wistar Iffa Credo, France	260–280 g	–				SSR125543	3–30	IP	60 min
4.2	UU	Mouse	DBA/2J Charles River, Germany	7–8 weeks	–				CP154,526	10–40	IP	30 min
5.1	UU	Mouse	CRFtg, wild-type[d]	9–16 weeks	MK801 hydrogen maleate	0.3; 1.0	IP	acute	–			
5.2	UU	Mouse	CRFtg, wild-type[d]	9–16 weeks	D-Amphetamine sulphate	2.5; 5.0	IP	10 min	–			
6	UU	Mouse	CRFtg, wild-type[d]	9–16 weeks	D-Amphetamine sulphate	5.0	IP	–	CP154,526	40	IP	–

UU Utrecht University, *i.t.i.* injection-test interval, *IP* intraperitoneal, *SC* subcutaneous, *NL* Netherlands

[a] All experiments were performed according to the Guide for Care and Use of Laboratory animals and were approved by the Ethical Committees for Animal Research of Utrecht University and Sanofi-Aventis

[b] Doses were based on dose–response pilots (not shown)

[c] Doses were based on previous experiments from our laboratories (Millan et al. 2001; Griebel et al. 2002; Groenink et al. 2008; see Table 2)

[d] CRFtg mice (line 2122, 17th generation, C57Bl/6J background) were generated as previously described (Dirks et al. 2002a). Wild-type littermates served as control mice

Drugs

(+)-MK801 hydrogen maleate (Sanofi Medicinal Chemistry, France, and Research Biochemicals Incorporated, USA, respectively), (±)-ketamine hydrochloride (Vetoquinol, Breda, the Netherlands) and D-amphetamine sulphate (Fagron BV, Nieuwerkerk a/d IJssel, the Netherlands) were dissolved in saline. CP154,526, DMP695 (gifts from Servier, Croissy/Seine France) and SSR125543 (gift from Sanofi, Paris, France) were suspended in a vehicle containing saline and Tween 80 (1 %). In studies 2.1 and 4.1, SSR125543 was suspended in saline.

Rationale for testing certain drugs and doses in particular species and strains was based on previous dose–response studies from our laboratories, in which significant behavioural or physiological effects were observed (for a summary, see Table 2). As shown in Table 2, CP154,526 and DMP695 improved PPI in CRFtg mice on a C57Bl/6J background. Effective doses from these studies were selected to test in other PPI-disruption models in C57Bl/6J mice. SSR125543 was proven effective in CD1 mice; hence, the effective dose was selected and tested on MK801-induced PPI disruption in CD1 mice. The minimal effective doses at which DMP695 and SSR125543 were found active in our rat anxiety tests were more consistent than the minimal effective dose for CP15,4526. Therefore DMP695 and SSR125543 were selected for the rat PPI studies.

Prepulse inhibition of the acoustic startle reflex

Apparatus and test procedure

Studies 1.1, 1.2, 2.2, 3.1, 3.2, and 4.2 Startle reflexes were measured in eight identical startle response systems (SR-LAB; San Diego Instruments, San Diego, CA, USA). Startle stimuli (115 dB, 50 ms) were presented alone, or preceded by noise prepulses (20 ms) of 2, 4, 8, or 16 dB above background (70 dB), with 100 ms between onsets of the prepulse and startle stimuli. The test session started with a 5-min acclimation period followed by three consecutive blocks of test trials (block 1 and 3, startle-stimulus alone trials; block 2, startle-stimulus alone, startle+prepulse, and no-stimulus trials). Intertrial intervals ranged from 10 to 20 s, and total test duration was 25 min.

Studies 2 1 and 4.1 Animals were tested in four startle boxes (Med Associates, East Fairfield, VT, USA). Startle pulses (120 dB, 50 ms) were preceded by prepulses (rats: 30 ms, mice: 20 ms) of 7, 14, or 20 dB above background (65 dB). Onsets of pulses and prepulses were separated by 100 (rats) or 40 (mice) ms. The test session started with a 5-min acclimation period followed by five startle stimuli that served to accustom the animals to the startle pulses. These startle pulses were

Table 2 Overview of dose–response studies from our laboratories, reporting behavioural or physiological effects of CRF_1 receptor antagonists in the dose range studied

	Subjects	CP15,4526			DMP695			SSR125543			Reference
Read-out	Mouse strain	Dose range	Route	i.t.i	Dose range	Route	i.t.i.	Dose range	Route	i.t.i.	
PPI	C57Bl/6 J	40 mg/kg	IP	30 min	40 mg/kg	IP	30 min	–	–	–	Present study
	DBA/2 J	10, 20, 40 mg/kg	IP	30 min	–	–	–	–	–	–	Present study
	CD1	–	–	–	–	–	–	10, 30 mg/kg	IP	60 min	Present study
PPI in CRFtg	C57Bl/6 J	20, **40**, **80** mg/kg	IP	30 min	10, 20, **40** mg/kg	IP	30 min	–	–	–	Groenink et al. 2008
Plasma corticosterone levels	C57Bl/6 J	**40** mg/kg	IP	30 min	**40** mg/kg	IP	30 min	–	–	–	Groenink et al. 2008
Stress-induced hyperthermia	C57Bl/6 J	**10**, **20**, **40** mg/kg	IP	60 min	**10**, **20**, **40** mg/kg	IP	60 min	–	–	–	Vinkers et al. 2012
Elevated plus maze (after social defeat)	CD1	–	–	–	–	–	–	**10**, **30** mg/kg	IP	60 min	Griebel et al. 2002
Read-out	Rat strain	Dose range	Route	i.t.i.	Dose range	Route	i.t.i.			Route	i.t.i.	
PPI	Wistar	–	–	–	40 mg/kg	IP	30 min	30 mg/kg	IP	30 min	Present study
	Wistar	–	–	–	–	–	–	3, 10, 30 mg/kg	IP	60 min	Present study
Vogel conflict	Wistar	5, 40, **80** mg/kg	IP	30 min	10, 20, **40** mg/kg	IP	30 min	–	–	–	Millan et al. 2001
	Wistar	–	–	–	–	–	–	10, **20**, **30** mg/kg	IP	60 min	Griebel et al. 2002
Social interaction	Sprague–Dawley	0.16, 1.25, **2.5**, 10 mg/kg	IP	30 min	2.5, 10, **40** mg/kg	SC	30 min	–	–	–	Millan et al. 2001

Data in bold reported significant effective dose

i.t.i. injection test interval

followed by a block of 40 stimuli, in which equal amounts of startle-stimulus alone, and startle + prepulse trials (with each of the three prepulse intensities), were presented in pseudorandom order. Intertrial intervals were variable (mice: 18–25 s, rats: 15–25 s). In study 2.1, mice were individually housed prior to the first injection.

Matching

One week before drug testing, or 1 day in study 4.1, a baseline PPI measurement was performed, in order to familiarize the subjects to the test procedure and to create treatment groups with equal mean percent PPI. In study 2.1, no matching session was included.

Microdialysis

Probe implantation Microdialysis probes were implanted in the mPFC (left probe, MAB 4.7.2. CU; AP +1.9, ML +0.9, DV −3.3 from bregma) and NAc (right probe, MAB 4.7.1. CU; AP +1.5, ML +1.0, DV −5.0 from bregma). Probes were secured with dental cement. To ensure that the cement would be held in place, shallow lines were carved into the skull. After microdialysis probe implantation, mice were housed individually for the duration of the experiment.

Experimental procedures Two days after implantation, microdialysis experiments were performed in conscious freely moving mice. First, the system was perfused with Ringer solution (147 mM NAcl, 2.3 nM KCl, 2.3 mM $CaCl_2$, and 1 mM $MgCl_2$) with the use of a KdScientific Pump 220 series (USA) at constant flow rate of 1 ml/min. Mice were connected to a dual channel swivel (type 375/D/22QM) which allowed them to move relatively unrestricted. During microdialysis, the pump rate was set at 0.07 ml/h. Two hours after connection, ten 30-min samples (i.e., samples 1 to11) were manually collected in vials containing 15 μl of 0.1 M acetic acid and frozen at −80 °C until analysis with HPLC. After 2 h of baseline samples (samples 1 to 4), mice were injected IP with CP154,526 (0, 40 mg/kg), followed by D-amphetamine (0, 5 mg kg) 30 min later, where after additional samples were collected for 3 h (samples 5 to11).

Histology

After 3 days, mice were sacrificed and their brains were quickly frozen in isopentane and stored at −80 °C. For probe localization, brains were transferred to a 30 % sucrose solution and after 2–3 days, frozen slices of 60 μm were made. These slices were stained with a cresyl violet staining for probe track verification. Data were discarded if the microdialysis probe was not in the PFC or NAc (one animal removed from the CRFtg/NAc-amphetamine group).

HPLC-ECD

For HPLC with electrochemical detection, an Alexyz 100 LC-EC system (Antec, The Netherlands) was used, consisting of two pumps, one auto-sampler with a ten-port injection valve, two columns and two detector cells. The mobile phase for column 1 (DA) consisted of 50 mM phosphoric acid, 8 mM KCl, 0.1 mM EDTA (pH 6.0), 12 % methanol and 500 mg/l 1-Octanesulfonic acid, sodium salt (OSA); and for column 2 (DOPAC) of 50 mM phosphoric acid, 50 mM citric acid, 8 mM KCl, 0.1 mM EDTA (pH 3.2), 10 % methanol and 500 mg/l OSA. From each microdialysis sample 5 μl was injected simultaneously onto each column. Mobile phases were pumped at 50 μl/min. Dopamine and the metabolites, DOPAC and HVA, were detected electrochemically using μVT-03 flow cells (Antec, the Netherlands) with glassy carbon working electrodes. Potential settings were for DA + 0.30 V, and for DOPAC +0.59 versus Ag/AgCl. The chromatogram was recorded and analysed using the Alexys data system. The limit of detection was 0.03 nM (S/N ratio 3:1). Methods have been described in detail elsewhere (Prins et al. 2011).

Statistics

Percent PPI was calculated as the mean startle magnitude to startle stimulus-alone, minus the mean startle magnitude to startle + prepulse stimuli, all divided by the mean startle stimulus-alone trials, and multiplied by 100. For calculation of the mean startle magnitude, only data from blocks that included prepulse trials were used. PPI data were analysed using repeated-measures analysis of variance (ANOVA) with prepulse intensity as within-subjects factor and CRF_1 antagonist and drug pretreatment (PPI disruption experiments), or CRF_1 receptor antagonist (naturally low PPI experiments) as between-subjects factor(s). PPI data were collapsed across prepulse intensities if drug effects were independent of prepulse intensity.

In study 2.1, the effect of SSR125543 on MK801-induced PPI disruptions, and the effects of MK801 and SSR125543 on PPI under vehicle conditions were analysed using three separate repeated-measures ANOVAs. In studies 1.1, 1.2 and 2.2, MK801 and D-amphetamine only disrupted PPI at 2, 4 and 8 dB prepulses, so treatment effects of the CRF_1 receptor antagonist were analysed for these intensities. Acoustic startle magnitude was analysed using one-way ANOVA with drug (pre-) treatment as between factor. Post-hoc analyses were performed by Dunnett's and Bonferroni corrected *t*-tests.

In the microdialysis experiment, data for mPFC and NAc, and for dopamine and DOPAC were analysed separately. Genotype differences in mean basal values were analysed using Student's *t*-test on the mean value of samples 1 to 4, from all animals under study. Data on combined drug

treatment were analysed using repeated-measures ANOVA with time (samples 5–11) as within-subjects factor and genotype and treatment as between-subjects factors, followed by separate repeated-measures ANOVAs for the three different treatment conditions. In the latter case, drug effects were analysed relative to mean basal values (samples 1 to 4), over a period of 2.5 h starting at 30 min post-injection (CP154, 526: samples 5 to 10; combined treatment CP154,526 and amphetamine: samples 6 to11). Post-hoc analyses for changes over time were analysed with simple contrasts relative to mean basal value. Post-hoc analysis for comparisons between genotypes at certain time points was performed with *t*-tests or multivariate ANOVA with basal and post injection samples as dependent and genotype as fixed factor. AUC was calculated using the trapezoid algorithm, for values from sample 4 (CP154526, or combined treatment) or sample 5 (D-amphetamine) onwards. AUC data were analysed by one-way ANOVA followed by Bonferroni-corrected *t*-tests.

The level of significance was set at $p<0.05$. Statistical analyses were carried out using SPSS for Windows, version 20.

Results

In every case reported here, percent PPI increased significantly with increasing prepulse intensity and this effect will not be described further. As drug treatment effects were independent of prepulse intensity in all cases, PPI was collapsed across intensity for the purpose of clarity. Data on the baseline startle response are summarized in Tables 3 and 4.

Experiment 1: effects of CRF_1 receptor antagonists in D-amphetamine disrupted PPI

SSR125543 in Wistar Wi rats

D-Amphetamine (2.5 mg/kg, IP) significantly disrupted PPI in Wistar Wi rats ($F_{1,44}=8.8$; $p=0.005$), independent of prepulse intensity ($F_{2,88}<1$, $p=0.997$). SSR125543 (30 mg/kg) did not alter D-amphetamine-induced PPI deficits (interaction D-amphetamine × CRF_1 antagonist, $F_{1,44}<1$; $p=0.9$), and SSR125543 had no effects on percent PPI by itself ($F_{1,44}<1$; $p=0.7$) (Fig. 1a).

CP154,526 in C57Bl/6J mice

D-Amphetamine (3.0 mg/kg, IP) significantly disrupted PPI in C57Bl/6J mice ($F_{1,50}=19.0$; $p<0.001$), independent of prepulse intensity ($F_{2,77}<1$, $p=0.9$). CP154,526 (40 mg/kg) did not significantly alter D-amphetamine-induced PPI deficits (interaction, D-amphetamine × CRF_1 antagonist, $F_{1,50}=2.5$; $p=0.12$), and CP154,526 had no overall effect on percent PPI ($F_{1,50}<1$; $p=0.8$) (Fig. 1b).

Experiment 2: effects of CRF_1 receptor antagonists in MK801 disrupted PPI

SSR125543 in CD1 mice

MK801 (0.2 mg/kg, IP) significantly disrupted PPI in CD1 mice ($F_{1,14}=8.5$; $p=0.01$), independent of prepulse intensity ($F_{2,28}<1$; $p=0.4$). SSR125543 (10, 30 mg/kg) did not significantly alter MK801-induced PPI deficits ($F_{2,21}<1$; $p=0.5$), and SSR125543 (30 mg/kg) had no significant effect on percent PPI by itself ($F_{1,14}<1$; $p=0.5$) (Fig. 2a).

DMP695 in C57Bl/6J mice

MK801 (0.5 mg/kg, IP) significantly disrupted PPI in C57Bl/6J mice ($F_{1,32}=9.1$; $p=0.005$), independent of prepulse intensity ($F_{2,64}<1$; $p=0.6$). DMP695 (40 mg/kg) did not significantly affect MK801-induced PPI deficits (interaction, MK801 × CRF_1-antagonist, $F_{1,32}<1$; $p=0.4$). DMP695 had no significant effects on percent PPI in C57Bl/6J mice per se (main effect DMP695, $F_{1,32}=1.4$; $p=0.2$) (Fig. 2b).

Experiment 3: effects of CRF_1 receptor antagonists in ketamine-disrupted PPI

DMP695 in Wistar Wu rats

Ketamine (5 mg/kg, SC) significantly disrupted PPI in Wistar Wu rats ($F_{1,43}=26.1$; $p<0.001$), dependent on prepulse intensity ($F_{2,86}=4.0$; $p=0.02$). However, further analysis showed that PPI was significantly disrupted at each prepulse intensity, so data were collapsed across intensities. DMP695 (40 mg/kg) did not significantly alter ketamine-induced PPI deficits (interaction, ketamine × CRF_1 antagonist, $F_{1,43}<1$; $p=0.5$). Also, DMP695 had no significant effect on percent PPI in Wistar rats on itself (main effect DMP695, $F_{1,43}=3.2$; $p=0.08$) (Fig. 3a).

CP154,526 in C57Bl/6J mice

Ketamine (30 mg/kg, IP) significantly disrupted PPI in C57Bl/6J mice ($F_{1,53}=19.3$; $p<0.001$), independent of prepulse intensity ($F_{2,106}=1.7$; $p=0.2$). CP154,426 (40 mg/kg) did not significantly alter ketamine-induced PPI deficits (interaction, ketamine × CRF_1 antagonist, $F_{1,53}=3.4$; $p=0.07$), and it had no overall effect on PPI in C57Bl/6J mice ($F_{1,53}<1$; $p=0.99$) (Fig. 3b).

Table 3 Drug effects on baseline startle response (expressed as mean±SEM)

Experiment	Startle amplitude (AU)			
	Vehicle–vehicle	Drug pretreatment	CRF_1 antagonist	Drug pretreatment + CRF_1 antagonist
1.1. D-Amph+SSR	1421±228	1060±99	1218±249	842±127
1.2. D-Amph+CP	303±35	352±86	293±73	214±52
2.1. MK801+SSR	567±77	389±53	262±48	(10) 491±82 (30) 429±59
2.2. MK801+DMP	264±45	439±71	344±64	318±47
3.1. ket+DMP	676±103	447±74	450±122	370±48
3.2. ket+CP	448±40	493±75	525±68	418±62
4.1. Wistar low — SSR	1817±54	–	(3) 1747±76 (10) 1695±61 (30) 1830±60	–
4.2. DBA low — CP	218±34	–	(10) 147±22 (20) 160±29 (40) 191±34	–
	Drug treatment	Startle amplitude (AU)		
		Wild-type	CRFtg	
5.1. CRFtg–MK801	0	336±38	387±66	
	0.3	509±82	410±52	
	1.0	571±93	508±86	
5.2. CRFtg–D-Amph	0	486±50	700±62	
	2.5	422±73	380±76	
	5.0	464±89	481±35	

D-Amph D-amphetamine, *SSR* SSR125543, *CP* CP154,526, *DMP* DMP695, *ket* ketamine, *AU* arbitrary unit, (*x*) drug dose

Experiment 4: effects of CRF1 receptor antagonists on spontaneously low PPI

SSR125543 in Wistar Iffa Credo rats

As shown in Fig. 4a, the effect of SSR125543 (3–30 mg/kg) on percent PPI in Wistar Iffa Credo rats was dependent on prepulse intensity ($F_{5,64}$=3.5; p=0.005). Subsequent post-hoc analysis however, did not show a significant treatment effect on percent PPI at any prepulse intensity. SSR125543 also did not significantly alter the mean percent PPI in these rats (see Fig. 4a, $F_{3,32}$<1; p=0.7).

CP154,526 in DBA/2J mice

CP154,526 (10–40 mg/kg) had no significant effect on percent PPI in DBA/2J mice ($F_{3,44}$=1.4; p=0.3), regardless of prepulse intensity (see Fig. 4b, $F_{9,132}$=1.1; p=0.3).

Table 4 Summary of the ANOVA results from startle data presented in Table 3

Experiment	Main effect drug pretreatment	Main effect CRF_1 antagonist	Interaction effect pretreatment × CRF_1 antagonist
1.1. D-Amph+SSR	$F_{1,44}$=3.9, p=0.06	$F_{1,44}$=1.3, p=0.3	$F_{1,44}$<1, p=0.97
1.2. D-Amph+CP	$F_{1,50}$<1, p=0.7	$F_{1,50}$=1.1, p=0.3	$F_{1,50}$<1, p=0.4
2.1. MK801+SSR	$F_{1,35}$<1, p=0.4	$F_{2,35}$=1.4, p=0.3	$F_{1,35}$<1, p=0.6
2.2. MK801+DMP	$F_{1,32}$=1.6, p=0.2	$F_{1,32}$<1, p=0.7	$F_{1,32}$=2.9, p=0.1
3.1. ket+DMP	$F_{1,44}$=2.9, p=0.1	$F_{1,44}$=2.8, p=0.1	$F_{1,44}$<1, p=0.4
3.2. ket+CP	$F_{1,53}$<1, p=0.7	$F_{1,53}$<1, p=0.7	$F_{1,53}$=1.5, p=0.2
4.1. Wistar low — SSR	–	$F_{2,32}$=2.4, p=0.08	–
4.2. DBA low — CP	–	$F_{3,48}$=1.1, p=0.3	–
Experiment	Main effect drug treatment	Main effect genotype	Interaction effect treatment × genotype
5.1. CRFtg–MK801	$F_{2,79}$=3.0, p=0.053	$F_{1,79}$<1, p=0.5	$F_{2,79}$<1, p=0.6
5.2. CRFtg–D-Amph	$F_{2,88}$=4.1, p=0.02*	$F_{1,88}$=1.0, p=0.3	$F_{2,88}$=1.9, p=0.15

D-Amph D-amphetamine, *SSR* SSR125543, *CP* CP154,526, *DMP* DMP695 *ket* ketamine

*p<0.05

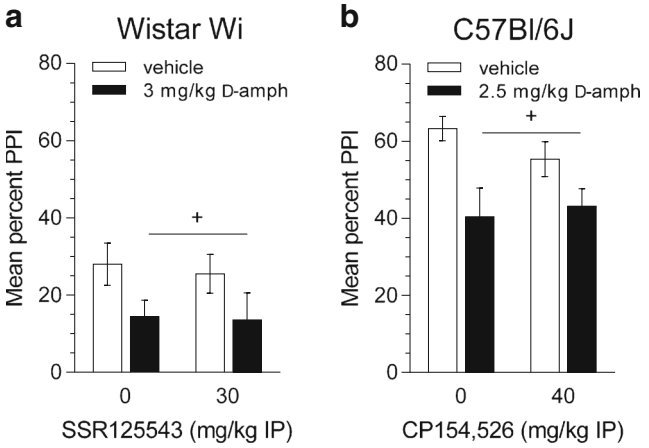

Fig. 1 Effects of CRF_1 receptor antagonists on D-amphetamine-disrupted PPI. **a** SSR125543 in Wistar Wi rats, **b** CP154,526 in C57Bl/6J mice. Data are collapsed over three prepulse intensities and expressed as mean±SEM. "+" depicts a main drug effect of D-amphetamine. Group sizes: **a** $n=12$; **b** vehicle–vehicle, D-amphetamine–CP154,526: $n=14$; CP154,526–vehicle: $n=13$; vehicle–D-amphetamine: $n=12$

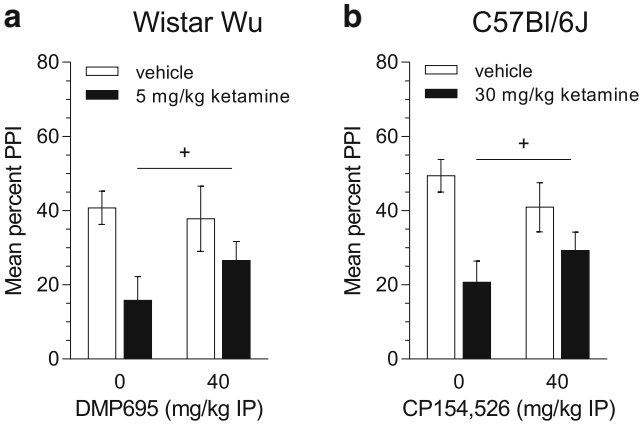

Fig. 3 Effects of CRF_1 receptor antagonists on ketamine-disrupted PPI. **a** DMP695 in Wistar Wu rats, **b** CP154,526 in C57Bl/6J mice. Data are collapsed across three prepulse intensities and expressed as mean±SEM. + depicts a main treatment effect of ketamine. Group sizes: **a** $n=12$; **b** vehicle–vehicle: $n=15$, other groups, $n=14$

Experiment 5: effects of chronically elevated CRF levels on sensitivity to D-amphetamine and MK801-disrupted PPI

MK801 in CRFtg mice

The effect of MK801 was dependent on genotype ($F_{2,79}=3.3$; $p=0.04$), and independent of prepulse intensity ($F_{5,237}<1$; $p=0.7$). Further analysis showed that MK801 had no effect in CRFtg mice ($F_{2,40}<1$; $p=0.5$), whereas it significantly disrupted PPI in wild-type mice both at 0.3 and 1.0 mg/kg ($F_{2,39}=13,2$; $p<0.001$). When comparing CRFtg and wild-type mice, percent PPI of the former was significantly lower for the vehicle and 0.3 mg/kg MK801 conditions, and similar at the highest dose of MK801 tested (Bonferroni corrected *t*-tests; Fig. 5a).

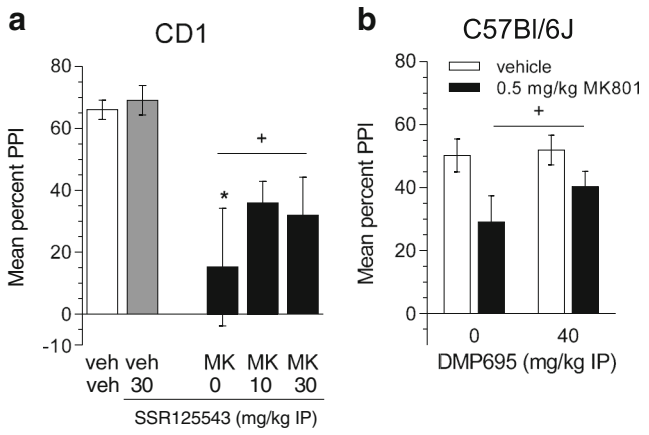

Fig. 2 Effects of CRF_1 receptor antagonists on MK801-disrupted PPI. **a** SSR125543 in CD1 mice; *veh* vehicle; MK, MK801, 0.2 mg/kg. **b** DMP695 in C57Bl/6 J mice. Data are collapsed over three prepulse intensities and expressed as mean±SEM. *$p<0.05$ compared to vehicle controls. "+" depicts a main treatment effect of MK801. Group sizes: **a** $n=8$; **b** vehicle–vehicle, DMP695–vehicle: $n=8$; vehicle–MK801, DMP695–MK801, $n=10$

D-Amphetamine in CRFtg mice

D-Amphetamine significantly disrupted PPI ($F_{2,86}=4.3$; $p=0.016$), independent of genotype ($F_{2,86}<1$; $p=0.99$) and prepulse intensity ($F_{4,258}=2.0$; $p=0.1$). Post-hoc Dunnett's test showed that the D-amphetamine-induced PPI disruptions were significant only for the 2.5 mg/kg dose. PPI in CRFtg mice was significantly lower than in wild-type mice for all treatment groups ($F_{1,86}=72$; $p<0.001$; Fig. 5b).

Experiment 6: effects of CP154,526 on extracellular dopamine following D-amphetamine treatment in CRFtg mice

Dopamine in NAc

Basal dopamine levels were similar in CRFtg and wild-type mice (Student's *t*-test, $p=0.3$) (Table 5).

The overall repeated-measures ANOVA on dopamine concentrations in NAc showed a significant treatment × time × genotype effect ($F_{3,138}=4.0$, $p=0.001$). Analysis per treatment showed that CP154,526 significantly increased extracellular dopamine concentrations, at each time point measured (post-hoc after significant ANOVA, $F_{6,48}=117$, $p<0.001$). As shown in Fig. 6a, this effect was similar for both genotypes ($F_{6,48}=2$; $p=0.08$).

D-amphetamine treatment significantly elevated dopamine concentrations, at each time point measured (post-hoc after significant ANOVA, $F_{1,48}=127$, $p<0.001$). This effect was dependent on genotype ($F_{1,48}=12$, $p=0.005$), and further analysis showed that the increase of extracellular dopamine

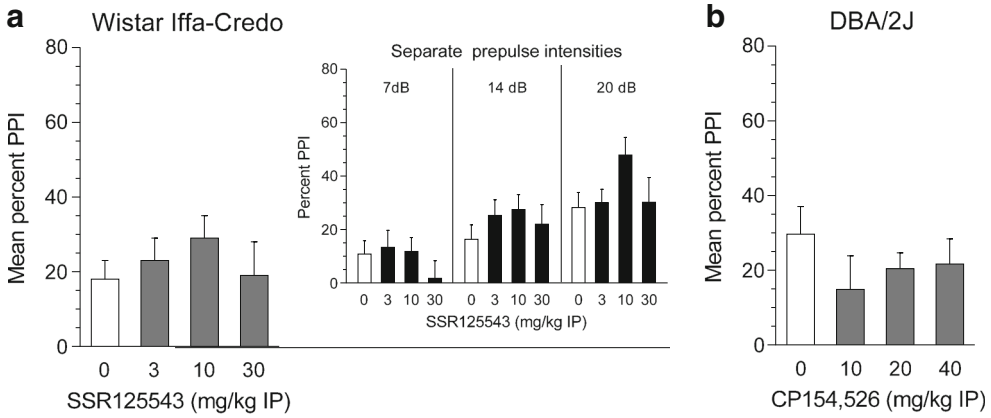

Fig. 4 Effects of CRF_1 receptor antagonists in models of spontaneously low PPI. **a** SSR125543 in Wistar Iffa Credo rats, **b** CP154,526 in DBA/2J mice. Data are collapsed across three (**a**) or four (**b**) prepulse intensities and expressed as mean±SEM. The insert in **a** shows the effect of SSR125543 at each prepulse intensity separately. Group sizes: **a** vehicle, SSR125543 (3 mg/kg): $n=10$; SSR125543 (10 mg/kg), $n=9$; SSR125543 (30 mg/kg) $n=7$; **b** all conditions $n=9$

by D-amphetamine was *lower* in CRFtg mice than in wild-types from 30 to 150 min after D-amphetamine injection (Fig. 6b).

Relative to mean basal dopamine levels, treatment with D-amphetamine preceded by CP154,526 injection resulted in a significant increase in dopamine concentrations in the NAc at each time point measured (post-hoc after significant ANOVA, $F_{1,42}=43$, $p<0.001$). Importantly, however, this effect was independent of genotype ($F_{2,42}<1$, $p=0.7$), indicating that CP154,526 abolished the differences between genotypes in the NAc with respect to the dopamine release after D-amphetamine treatment (Fig. 6c). In fact, apparently, CP154,526 increased the amphetamine-induced elevation in dopamine levels in CRFtg mice, while decreasing those levels in wild-types.

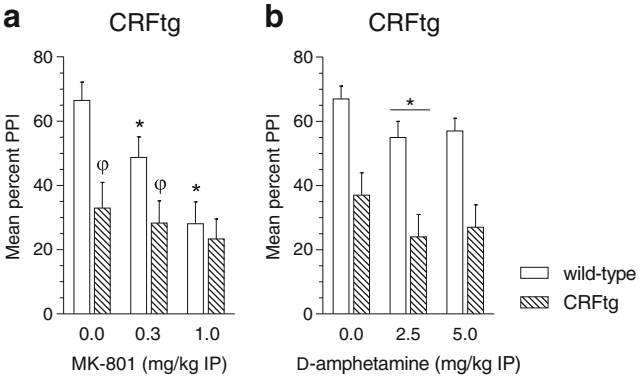

Fig. 5 Effects of **a** MK801 and **b** D-amphetamine on percent PPI in CRFtg and wild-type mice. Data are collapsed across four prepulse intensities and expressed as mean±SEM. *$p<0.05$ compared to corresponding vehicle, $^{\varphi}p<0.05$, genotype difference within treatment condition. Main genotype effects are not depicted. + depicts a main treatment effect of D-amphetamine at 2.5 mg/kg. Group sizes: **a** vehicle and MK801 (0.3 mg/kg) condition: wild-type $n=14$, CRFtg $n=14$; for MK801 (1.0 mg/kg): wild-type $n=14$, CRFtg: $n=15$; **b** for vehicle and D-amphetamine (2.5 mg/kg) wild-type $n=17$, CRFtg: $n=13$; D-amphetamine (5.0 mg/kg): wild-type $n=18$, CRFtg: $n=14$

In Table 6, the AUC values are given for NAc dopamine concentrations following treatment with CP154,526, D-amphetamine or the combination of both agents in CRFtg and wild-type mice. Univariate ANOVA showed that there was a significant genotype × treatment interaction ($F_{2,23}=4.2$; $p=0.027$). According to post-hoc *t*-tests, D-amphetamine-induced dopamine overflow was significantly lower in CRFtg than in wild-type mice, and CP154,526 significantly increased the amount of dopamine released by D-amphetamine in CRFtg but did not alter the D-amphetamine effect in wild-types.

DOPAC in NAc

Basal DOPAC levels were similar in both genotypes (Student's *t*-test, $p=0.8$).

D-amphetamine significantly reduced NAc extracellular DOPAC levels, measured as AUC (post-hoc after significant ANOVA ($F_{2,22}=52$, $p<0.001$). This treatment effect was similar for both genotypes ($F_{2,22}<1$, $p=0.7$), and unaltered by CP154,526 (Table 7).

Dopamine in mPFC

Basal dopamine levels were similar in wild-type and CRFtg mice (Student's *t*-test, $p=0.3$) (Table 5).

The overall repeated-measures analysis on dopamine levels in the mPFC showed a significant treatment × time × genotype effect ($F_{12,156}=3.4$, $p<0.001$).

Further analysis showed that treatment with CP154,526 elevated extracellular dopamine concentrations over time ($F_{6,48}=23$, $p<0.001$) compared to baseline, which was significant from 60 to 120 min after injection (post-hoc). The effects of CP154,526 on dopamine concentrations were comparable for both genotypes ($F_{6,48}=1.8$, $p=0.11$) (Fig. 6d).

Table 5 Basal levels of extracellular dopamine, DOPAC and HVA, in CRFtg and wild-type mice

	Nucleus accumbens		Medial prefrontal cortex	
	Wild-type ($n=15$)	CRFtg ($n=14$)	Wild-type ($n=14$)	CRFtg ($n=18$)
Dopamine (nM)	1.9±0.1	1.5±0.2	0.3±0.03	0.2±0.02
DOPAC (nM)	248±15.0	223±22.6	42.6±5.8	29.1±2.5 *
HVA (nM)	218±8.8	235±18.3	68.9±17.6	72.1±12.9

*$p<0.01$, compared to corresponding wild-type

Treatment with D-amphetamine increased extracellular dopamine concentrations in the mPFC ($F_{6,48}=81$, $p<0.001$), which was significant for all time points (post-hoc). The changes observed over time were dependent on genotype ($F_{6,48}=9.8$, $p<0.001$). Further analysis indicated that, similar to the NAc, peak dopamine concentrations were significantly lower in CRFtg mice than in wild-types, at 30 min post injection (post-hoc) (Fig. 6e).

Treatment with D-amphetamine preceded by CP154,526 injection resulted in a significant increase in dopamine concentrations at each time point measured, relative to mean basal dopamine levels (post-hoc following significant ANOVA, $F_{6,60}=39.8$, $p<0.001$). This effect of combined treatment was independent of genotype ($F_{6,60}<1$, $p=0.9$), indicating that CP154,526 abolished the differences between genotypes in the mPFC with respect to dopamine response after D-amphetamine treatment (Fig. 6f).

Table 6 depicts the AUC values of dopamine released after treatment with CP154,526, D-amphetamine or the combination of both agents, in CRFtg and wild-type mice.

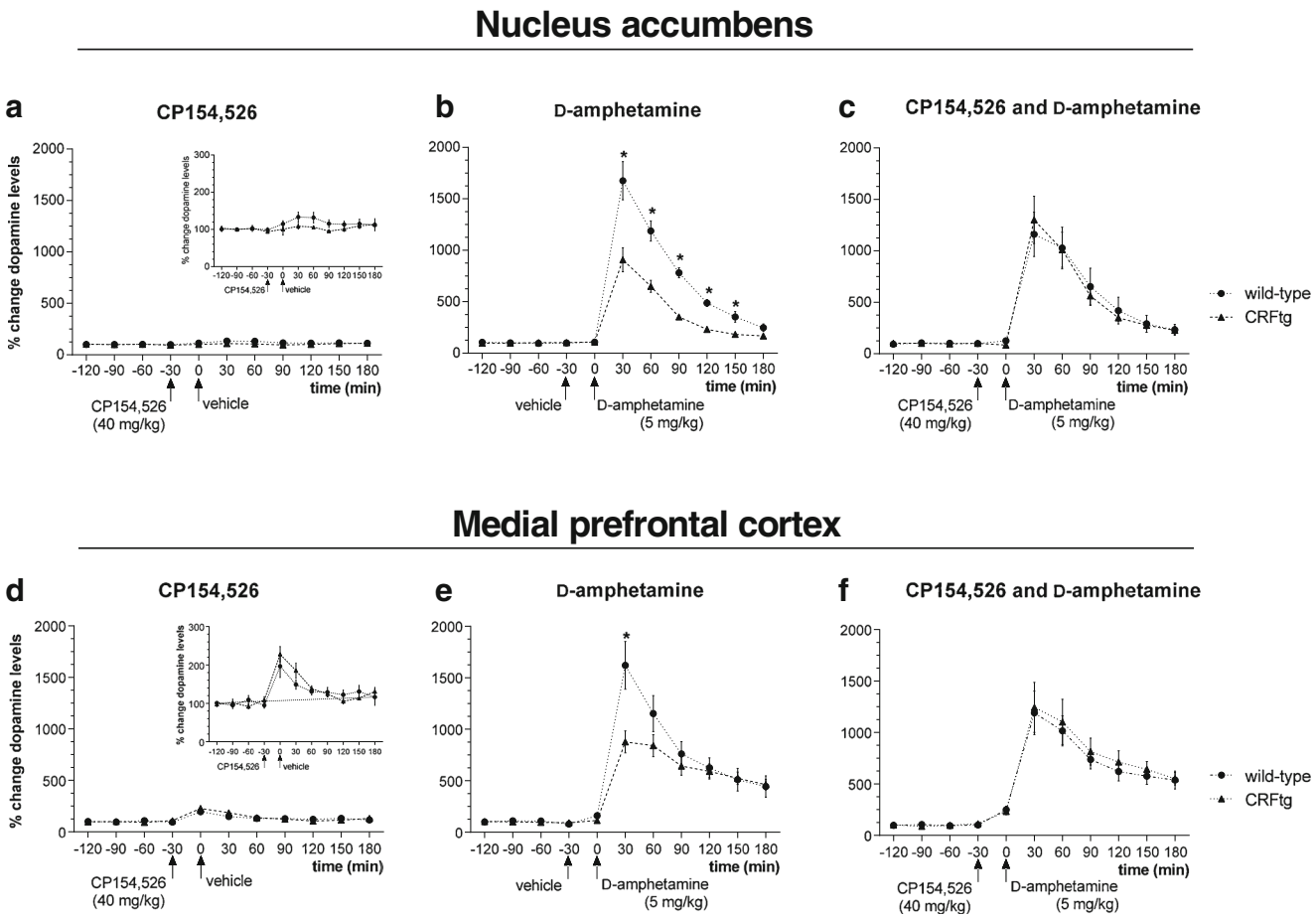

Fig. 6 Effect of treatment with CP154,526 (at $t=-30$ min) (**a**, **d**), D-amphetamine (at $t=0$ min) (**b**, **e**) or combination of CP154,526 and D-amphetamine (**c**, **f**) on changes in extracellular dopamine levels in wild-type and CRFtg mice. **a**–c Nucleus accumbens (*NAc*), **d**–**f** medial prefrontal cortex (mPFC). Time points −120 to −30 min represent baseline measurements. Group sizes: NAc, combined treatment in CRFtg mice $n=4$, all other groups $n=5$; mPFC, D-amphetamine in CRFtg mice, combined treatment in CRFtg and wild-type mice $n=6$; CP154,526 in wild-type, CRFtg mice $n=5$, D-amphetamine in wild-type mice $n=4$. Data are expressed as percentage of baseline±SEM. *$p<0.05$, genotype difference within drug condition

Table 6 Dopamine release (area under the curve (AUC), nM × min) after treatment with CP154,526 (40 mg/kg), D-amphetamine (5 mg/kg), or CP154,526 (40 mg/kg)+D-amphetamine (5 mg/kg) in CRFtg and wild-type mice

	Nucleus accumbens		Medial prefrontal cortex	
	Wild-type	CRFtg	Wild-type	CRFtg
CP154,526	114.7±37.5	61.2±16.5	180.8±44.0	209.8±26.7
D-Amphetamine	2082.9±205.4	982.0±91.0 *	2252.2±347.4	1636.0±228.6
CP154,526+D-amphetamine	1620.4±397.1	1573.5±244.8#	2054.8±338.5	2244.7±403.9

*$p<0.05$, relative to corresponding wild-type group

#$p<0.05$, relative to corresponding D-amphetamine group

In the mPFC, the amount of extracellular dopamine was not significantly influenced by any of the agents in either genotype ($F_{2,26}$=<1, $p=0.4$, N.S.).

DOPAC in mPFC

Basal DOPAC levels were significantly higher in wild-type than in CRFtg mice (Student's t-test, $p=0.009$).

D-Amphetamine significantly reduced mPFC extracellular DOPAC levels, measured as AUC (post-hoc after significant ANOVA ($F_{2,26}=66.7$, $p<0.001$). This treatment effect of D-amphetamine was similar in both genotypes ($F_{2,26}<1$, $p=0.9$), and unaltered by CP154,526 (Table 7).

Discussion

In this study we evaluated the effects of the non-peptidergic CRF_1 receptor antagonists CP154,426 (10–40 mg/kg), SSR125543 (3–30 mg/kg) and DMP695 (40 mg/kg) in rodent tests of disrupted PPI. CRF_1 receptor antagonists did not improve PPI disruption induced by D-amphetamine, by NMDA receptor antagonists, or in animal strains displaying spontaneously low PPI. As drugs were tested at doses and under conditions demonstrated previously to be pharmacologically active (see Table 2, Millan et al. 2001; Griebel et al. 2002; Groenink et al. 2008) and as multiple and chemically distinct CRF_1 receptor antagonists yielded similar data in our study, current findings indicate that CRF_1 receptor blockade does not improve sensorimotor gating.

Table 7 DOPAC release (area under the curve (AUC), nM × min) after treatment with CP154,526 (40 mg/kg), D-amphetamine (5 mg/kg), or CP154,526 (40 mg/kg)+D-amphetamine (5 mg/kg) in CRFtg and wild-type mice

	Nucleus accumbens		Medial prefrontal cortex	
	Wild-type	CRFtg	Wild-type	CRFtg
CP154,526	54.2±31.3	10.2±20.0	150.9±23.8	188.1±19.1
D-amphetamine	−124.6±8.1	−140.5±8.9	−81.7±2.1	−54.9±8.2
CP154,526+D-amphetamine	−102.6±18.1	−143.7±6.7	−7.8±15.1	13.6±29.8

Group sizes: nucleus accumbens (wild-type, CRFtg; CP154,526, D-amphetamine, and combined treatment, each group $n=5$; CRFtg, combined treatment, $n=4$); Prefrontal cortex (CP154,526 in wild-type, CRFtg; $n=5$; D-amphetamine in wild-type, $n=4$; combined treatment in CRFtg and D-amphetamine in wild-type and CRFtg, $n=6$)

CRF_1 receptor antagonists and dopamine interactions

To our knowledge, this is the first study reporting on the effects of CRF_1 receptor antagonists in dopaminergic models of disrupted PPI. We found that CP154,526 (40 mg/kg) and SSR125543 (30 mg/kg) had no effect on PPI disruptions induced by the dopamine releaser D-amphetamine. D-Amphetamine-induced hyper locomotion, another read-out associated with antipsychotic potential, was also not reversed by CRF_1 receptor blockade (Giardino et al. 2012). A few PPI studies investigated the role of dopaminergic activation in CRF-induced PPI deficits, and found that haloperidol attenuates the PPI deficits of CRFtg mice and intracerebroventricular (ICV) CRF infusion in Wistar Kyoto rats (Dirks et al. 2003; Conti et al. 2005). These findings may suggest that CRF-induced PPI disruption involves enhanced dopaminergic activity, although studies in knock-out mice suggest that neither dopamine D_1 nor D_2 receptors are necessary for the CRF-induced PPI effects (Vinkers et al. 2007).

The PPI-disruptive effects of direct and indirect dopaminergic agonists have been linked to dopaminergic hyperactivity in the NAc (Wan et al. 1995; Swerdlow et al. 2001). In this key area of the mesolimbic dopamine system, CRF has been shown to modulate behaviours that involve dopaminergic neurotransmission. CRF facilitated cue-elicited motivation (Pecina et al. 2006) and social bonding (Lim et al. 2007), both behaviours thought to be dopamine dependent (Aragona et al. 2006; Lex and Hauber 2008). With respect to drug addiction – a composite behaviour that involves changes in mesolimbic dopamine pathways – CRF_1 receptor antagonists blocked drug withdrawal effects and stress-induced reinstatement of cocaine-seeking, a process related to onset of psychosis (Koob 2010; Blacktop et al. 2011; Almela et al. 2012). Considering the above described effects of CRF_1 receptor antagonists on behaviours that involve accumbal dopaminergic neurotransmission, the absence of effect of CRF_1 receptor antagonists on D-amphetamine induced PPI disruption suggests that acute blockade of CRF_1 receptors has limited effects on modulating or compensating dopaminergic hyperactivity related to sensory gating.

Our microdialysis study partly supports this notion, as the effects of CP154,526 on D-amphetamine-induced extracellular dopamine concentrations appeared dependent on the basal tone of the CRF system. CP154,526 did not alter D-amphetamine-induced rises in extracellular dopamine concentrations in wild-type mice. In CRFtg mice, however, prior treatment with CP154,526 reversed the reduced D-amphetamine-induced dopamine overflow, an effect that was more marked in NAc than in mPFC. To our knowledge, this is the first microdialysis study reporting on the interaction between a CRF_1 receptor antagonist and D-amphetamine. The attenuated extracellular dopamine response to D-amphetamine in CRFtg mice is likely a result of decreased intracellular dopamine stores, as D-amphetamine acts as a false substrate for the dopamine transporter and increases dopamine release by reversing transport to expel intra-terminal dopamine stores (Fleckenstein et al. 2007).The differential dopaminergic response of CRFtg and wild-type mice to D-amphetamine is however not reflected at the behavioural level, as wild-type and CRFtg mice were equally sensitive to the PPI disruptive effect of D-amphetamine. This discrepancy between neurochemical and behavioural effects is most likely explained by the fact that PPI is not solely determined by dopaminergic neurotransmission in the Nac, but involves other processes and brain areas as well (for a review, see Koch 1999).The finding that wild-type and CRFtg mice have similar basal dopamine levels in NAc and mPFC, shows that exposure to long-term elevated central CRF levels does not alter basal dopamine release in the mesocorticolimbic system. It may also suggest that the sensorimotor gating deficits in these CRFtg mice (Dirks et al. 2002b) are not directly related to enhanced dopaminergic activity, although the PPI deficits in CRFtg mice can be reversed with haloperidol (Dirks et al. 2003).

Finally, in the present study systemic administration of CP154,526 enhanced basal dopamine concentrations in mPFC and to a lesser extent in NAc. In rat studies, CP154,526 administration was without effect on baseline dopamine levels in PFC and NAc (Isogawa et al. 2000; Millan et al. 2001; Lu et al. 2003; Gurkovskaya et al. 2005). It is unclear what may have caused these different results.

CRF_1 receptor antagonists and NMDA-glutamate interactions

DMP695 (40 mg/kg), CP154,526 (40 mg/kg) and SSR125543 (30 mg/kg) had no significant effect on PPI disruptions induced by the NMDA receptor antagonists MK801 and ketamine.

We are not aware of other studies having reported on the effects of CRF_1 receptor antagonists in these tests. Systemic administration of NMDA receptor antagonists may affect multiple brain areas. Both the hippocampus and basolateral amygdala have been implicated in the PPI disruptive effects of NMDA receptor blockade, and interactions between CRF and glutamatergic systems relevant for sensorimotor gating may occur at these sites (Chen et al. 2004; Rainnie et al. 2004; Fu et al. 2007; Philbert et al. 2013). Infusion of MK801 in rat hippocampus induces profound PPI reductions (Bakshi and Geyer 1998, 1999; Bast et al. 2000). As CRF dose-dependently inhibits NMDA-induced currents in hippocampal neurons via a CRF_1 receptor dependent mechanism (Chen et al. 2004), this could be a potential site of interaction.

Local infusion of MK801 into the basolateral amygdala also disrupts PPI (Bakshi and Geyer, 1998, 1999; Fendt et al. 2000), an effect thought to be mediated via blockade of NMDA receptors on inhibitory interneurons, which may result in excitation of the basolateral amygdala output neurons (Benes 2010; Fendt et al. 2000). Besides this GABA-mediated disinhibition, CRF-mediated excitation is also implicated in acute glutamate receptor activation, which in turn may induce long-term synaptic plasticity and increase excitability of basolateral amygdala neurons (as reviewed by Shekhar et al. 2005). Interesting in this respect is our finding that CRFtg mice are less sensitive to the PPI-disruptive effects of MK801 than wild-type mice. Although it cannot be excluded that this genotype difference is caused by a floor effect, it may suggest that chronically elevated CRF levels alter NMDA–glutamate neurotransmission resulting in sensorimotor gating deficits, a process comparable to that described for emotional disorders (Rainnie et al. 2004).

The absence of effect of CRF_1 receptor antagonists on the PPI-disruptive effects of MK801 and ketamine in the present study, shows that acute blockade of CRF_1 receptor signalling does not improve PPI disruptions induced by NMDA receptor blockade. Although it cannot be excluded that *chronic* pharmacological blockade of the CRF_1 receptor would have beneficial effects, the acute effect of CRF_1 receptor antagonists on PPI deficits in CRFtg mice does not support this notion (Groenink et al. 2008).

CRF_1 receptor antagonists and spontaneously low PPI

SSR125543 (3–30 mg/kg) and CP154,526 (10–40 mg/kg) had no effect on the spontaneously low PPI response of Wistar Iffa Credo rats and DBA/2J mice, respectively. PPI in Wistar Iffa Credo rats has not broadly been characterized; some atypical antipsychotics, including clozapine, olanzapine, but not risperidone or the typical antipsychotic haloperidol improved PPI in this strain (Depoortere et al. 1997). DBA mice on the other hand, have been tested extensively in PPI (Olivier et al. 2001). Their low PPI response was improved by pharmacologically diverse compounds, including typical and atypical antipsychotics, mood stabilizers, metabotropic glutamate receptor ligands and glycine transporter inhibitors (Boulay et al. 2008; Flood et al. 2009; Hikichi et al. 2010; Flood et al. 2011). As with Wistar Iffa Credo rats, the neurochemical basis of the low PPI response of DBA/2J mice

is not understood; however, based on the current results, a role for increased CRF signalling is not likely to be involved.

Conclusions

In conclusion, we tested three different non-peptidergic CRF_1 receptor antagonists, using different PPI tests, in both mice and rats. CRF_1 receptor antagonists were without effect in any of the PPI tests, suggesting that CRF_1 receptors are not key modulators of sensorimotor gating, despite the fact that these receptors can affect PPI under conditions of high CRF tone.

We further showed that in CRFtg mice D-amphetamine-induced dopamine release is reduced, and that CRF_1 receptor antagonists may normalize this reduced dopamine outflow. In addition, we showed that CRF overexpressing mice are less sensitive to MK801-induced PPI disruption. These findings indicate that under conditions of enhanced CRF activity changes may occur in both dopaminergic and glutamatergic neurotransmission, which could be relevant for sensorimotor gating. As chronic stress has been implicated in the development of schizophrenia, and chronic stress may result in enhanced activity of CRF systems, current findings do not exclude the possibility that CRF_1 receptor antagonists, alone or in combination with antipsychotics, could be useful in improving sensorimotor gating under chronic stress conditions.

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