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Prefrontal GABA_B Receptor Activation Attenuates Phencyclidine-Induced Impairments of Prepulse Inhibition: Involvement of Nitric Oxide

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Recent theories propose that both GABA and glutamate signaling are compromised in patients with schizophrenia. These deficits can be observed in several brain regions including the prefrontal cortex (PFC), an area extensively linked to the cognitive dysfunction in this disease and notably affected by NMDA receptor antagonists such as phencyclidine (PCP). We have previously demonstrated that inhibition of the nitric oxide (NO) pathways in the brain, particularly in the PFC, prevents a wide range of PCP-induced behavioral deficits including disruption of prepulse inhibition (PPI). This study investigated the role of GABA_B receptor signaling and NO in the effects of PCP on PPI. Mice received systemic or prefrontal injections of the GABA_B receptor agonist baclofen (2.5–5 mg/kg and 1 mM) before PCP treatment (5 mg/kg) and were thereafter tested for PPI. GABA/NO interactions were studied by combining baclofen and the NO synthase inhibitor L-NAME (20 mg/kg) in subthreshold doses. The role of GABA_B receptors for NO production *in vivo* was assessed using NO-sensors implanted into the rat PFC. PCP-induced PPI deficits were attenuated in an additive manner by systemic baclofen treatment, whereas prefrontal microinjections of baclofen completely blocked the effects of PCP, without affecting PPI *per se*. The combination of baclofen and L-NAME was more effective in preventing the effects of PCP than any compound by itself. Additionally, baclofen decreased NO release in the PFC in a dose-related manner. This study proposes a role for GABA_B receptor signaling in the effects of PCP, with altered NO levels as a downstream consequence. Thus, prefrontal NO signaling mirrors an altered level of cortical inhibition that may be of importance for information processing deficits in schizophrenia.

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INTRODUCTION

Inhibitory neurotransmission appears to be impaired in patients with schizophrenia as evidenced by the large number of clinical studies implicating deficits in the GABA system. These deficits range from reduced amounts of mRNA coding both for the main GABA-generating enzyme, glutamic acid decarboxylase (GAD₆₇) and the GABA membrane transporter GAT-1 (for review see Hashimoto *et al*, 2008), to lowered levels of GABA (Cross *et al*, 1979; Perry *et al*, 1979) and a modestly decreased density of GABAergic interneurons (Benes *et al*, 1991, 2001). In addition, signs of such a hypofunction have been observed

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in many brain regions including areas that are considered central to the pathophysiology of schizophrenia such as the hippocampus (Benes, 1999), the anterior cingulate cortex (Woo *et al*, 2004) and the prefrontal cortex (PFC) (Akbarian *et al*, 1995; Hashimoto *et al*, 2003).

In parallel, excitatory glutamate receptors have been implicated in the pathophysiology of schizophrenia. This is based both on post-mortem studies showing changes in binding, transcription, and subunit expression of these receptors, and on altered levels of glutamate-related amino acids such as *N*-acetylaspartate and *N*-acetylaspartylglutamate (for review see Lewis and Moghaddam, 2006). In addition, noncompetitive NMDA receptor antagonists such as phencyclidine (PCP) induce behavioral changes in humans that resemble positive symptoms, negative symptoms, and cognitive deficits in schizophrenia (Freedman *et al*, 1987; Javitt and Zukin, 1991; Luby *et al*, 1959; Yesavage and Freman, 1978). The strategy of modeling aspects of schizophrenia in laboratory animals, by administration of noncompetitive NMDA antagonists such as PCP, MK-801, or ketamine has proven effective in mimicking

certain features of schizophrenia including disrupted working memory (Jentsch *et al*, 1997; Wass *et al*, 2006b) and impaired preattentive information processing as measured by prepulse inhibition of the acoustic startle (PPI; Fejgin *et al*, 2007; Klamer *et al*, 2001; Linn *et al*, 2003; Mansbach and Geyer, 1989). In the light of this, NMDA receptor hypofunction has been suggested as a potential mechanism underlying certain aspects of schizophrenia.

Interestingly, both clinical and animal studies show that administration of NMDA antagonists produces signs of an increase, rather than an expected decrease, in cortical activity (Breier *et al*, 1997; Gozzi *et al*, 2008; Jackson *et al*, 2004; Lahti *et al*, 1995; Moghaddam *et al*, 1997). These findings have led to the proposition that NMDA receptors situated on GABAergic interneurons may be affected by this class of psychotomimetic substances (Homayoun and Moghaddam, 2007). NMDA channels are known to be especially important for interneuron activity (Grunze *et al*, 1996; Jones and Buhl, 1993) and a recent study of PFC function shows that inhibition of NMDA receptors preferentially decreases the activity of fast-spiking interneurons, thereby causing a disinhibition of pyramidal cells (Homayoun and Moghaddam, 2007). In line with this, a recent publication by Behrens *et al* showed that the noncompetitive NMDA receptor antagonist, ketamine, induces a dysfunction of cortical interneurons that can be rescued by a GABA_A agonist (Behrens *et al*, 2007) and several studies have demonstrated deleterious effects of NMDA receptor antagonists on interneuron integrity and function. Taken together, this points to a possible interaction where glutamatergic output is disinhibited and subsequently increased as a consequence of a disrupted GABA system (for review see Lisman *et al*, 2008).

We have previously shown that the signaling molecule nitric oxide (NO) is important for the ability of PCP to induce schizophrenia-like behavior in rodents ranging from PPI to working memory (Klamer *et al*, 2004, 2005a; Palsson *et al*, 2007; Wass *et al*, 2006a, 2008). More specifically, an NO/cGMP signaling mechanism in the medial PFC appears to be important for these effects as cGMP, the main effector of NO signaling, is increased in this region following PCP treatment and normalized by pretreatment with a NO synthase (NOS) inhibitor. Furthermore, prefrontal microinjections with an inhibitor of cGMP synthesis blocked the effects of PCP on PPI (Fejgin *et al*, 2008). These findings indicate that elevated levels of NO in the PFC may be important for the effects of PCP. A possible explanation for such an increase in NO production following NMDA receptor antagonist treatment, could be a higher prefrontal glutamate efflux caused by inhibition of interneuron output followed by a subsequent disinhibition of glutamate release. Tentatively, this increase in glutamate levels may activate non-NMDA glutamate receptors and thereby induce NO release. Notably, NO-producing cells in the rodent medial PFC, hippocampus, and the primate frontal cortex also synthesize GABA (Gabbott and Bacon, 1995; Szabadits *et al*, 2007; Yan *et al*, 1996), indicating a close relationship between NO and GABA signaling. The GABA_B agonist baclofen has been shown to block the PPI deficits induced by the NMDA receptor antagonist MK-801 in both mice and rats, and also to improve spontaneous PPI deficits in DBA/2J mice (Arai *et al*, 2008; Bortolato *et al*, 2004, 2007). These

findings demonstrate that GABA_B signaling is important for the regulation of PPI and that the acute effects of NMDA antagonists may be mediated by disruption of interneuron function.

The aim of this study was to investigate the importance of GABA_B and NO signaling, particularly in the PFC, for the disruptive effects of PCP on preattentive information processing. To this end, mice received both systemic and prefrontal injections of the GABA_B receptor agonist baclofen before PCP treatment and were thereafter tested for PPI. To study GABA/NO interactions in relation to PPI, a combination of baclofen and the NOS inhibitor L-NAME were administered in doses not sufficient to block the effects of PCP *per se*. Finally, the regulatory role of GABA_B receptors for NO production was assessed *in vivo* using novel, microelectrochemical NO-sensors implanted into the rat medial PFC.

METHODS

Animals

Male NMRI mice (Charles River, Germany or B&K, Sweden; 25–35 g) and male Sprague–Dawley rats (Taconic, Denmark; 290–400 g) were used in this study. The animals were housed nine per cage (mice) or four (rats) per cage, in a colony room under constant temperature ($20 \pm 1^\circ C$) and humidity ($50 \pm 5\%$). Animals were allowed to acclimatize for at least 1 week before surgery or behavioral testing. The daylight cycle was maintained artificially (lights on from 0600 to 1800 hours) and behavioral experiments and biochemical measurements were conducted during the light phase. The Ethics Committee for Animal Experiments, Göteborg, Sweden, approved all experimental procedures used in this study.

Drugs

Phencyclidine hydrochloride (Sigma Chemicals, St Louis, MO, USA), R-baclofen (more active enantiomer; Sigma-Aldrich, Germany) and N^G-nitro-L-arginine methyl ester (L-NAME; Sigma-Aldrich) were used in this study. All drugs for systemic injections were dissolved in saline (0.9% NaCl) and administered either intraperitoneally (i.p., mice) or subcutaneously (s.c., rats). Mice were always injected a volume of 10 ml/kg and rats 2 ml/kg. For local injections, R-baclofen was dissolved in Ringer's solution. The doses were chosen for optimal disruption of PPI (PCP 5 mg/kg) and below the threshold for reversing these deficits (L-NAME) based on previously published studies (Klamer *et al*, 2001). Baclofen doses were based on PPI studies in mice (Arai *et al*, 2008; Bortolato *et al*, 2007) and rats (Bortolato *et al*, 2004).

Surgical Procedure

Local injection experiments. The mice were anesthetized with isoflurane (Isofluran Baxter; Apoteket AB, Sweden), placed in a Kopf stereotaxic instrument (David Kopf Instruments, Tujunga, CA, USA), and kept on a heating pad to prevent hypothermia and administered ketoprofen (Romefen, Veter, Sweden) to reduce postoperative pain. The

skull was then exposed, and two holes for the guide cannulas (stainless steel, length 10 mm, with an o.d./i.d. of 0.6/0.45 mm), and one hole for an anchor screw were drilled. The coordinates used for the medial PFC region relative to the bregma were as follows: anterior +1.8 mm, lateral to midline ±0.8 mm, and ventral 1.0 mm from the brain surface (Franklin and Paxinos, 1996). The dura was removed using a sharp needle, and the guide cannulas and the anchor screw were secured with dental cement (Dentalon plus; AgnTho's AB, Lidingö, Sweden). After surgery the mice were administered 1 ml of saline (s.c.) to prevent dehydration and allowed to recover for 3–4 days while housed individually in standard plastic cages.

NO measurements. The rats were anesthetized with isoflurane, placed in a Kopf stereotaxic instrument and kept on a heating pad to prevent hypothermia. An incision was placed down the midline of the skull and the bone was exposed. Four holes for the anchor screws, two holes for the reference (8T Ag wire, 200-µm bare diameter; Advent Research Materials, UK) and auxiliary (8T Ag wire) electrodes, and one hole for the sensor electrode was drilled. Electrodes were then implanted following a previously described procedure (Lowry *et al*, 1997). The coordinates used for the medial PFC relative to bregma were as follows: anterior +3.2 mm, lateral to midline ±0.8 mm, and ventral −4.2 mm from the brain surface. The electrode was inserted into the brain and connected to a pedestal that was secured to the anchor screws with dental cement. Sensor placement was balanced between the left and right hemispheres throughout the experiment. During surgery, the rats were administered 2.0 ml of saline (s.c.), to reduce postoperative dehydration and an analgesic (carprofen) to reduce postoperative pain. The animals were allowed to recover for 2–4 days before commencing experiments. They were housed individually in standard plastic cages.

Probe and injection placement verification. After termination of the local injection and voltammetry experiments the mice and rats were decapitated. The brains were removed and frozen at −80°C. Injection or sensor placement was verified by sectioning the brains using a cryostat and an atlas of the mouse or rat brain for reference (Figure 1; Franklin and Paxinos, 1996; Paxinos and Watson, 2005).

Prepulse Inhibition

Apparatus. Acoustic startle was recorded using a MOPS 3 startle response recording system (Metod och Produkt Svenska AB, Sweden). The animals were placed in small plexiglass cages (10 × 5.5 × 6 cm³) that were suspended at the top to a piston. A movement of the animal in the cage caused a displacement of the piston, the acceleration of which was registered by a piezoelectric accelerometer. This signal was sampled and digitized by a microcomputer that also controlled the delivery of acoustic stimuli. Startle amplitude was defined as the maximum signal amplitude occurring 8–30 ms after the startle-eliciting stimulus. Four cages were used simultaneously and each cage was housed in a dimly lit and sound-attenuated cabinet (52 × 42 × 38 cm³). The cages were calibrated for equal sensitivity before testing and a mouse was always tested in
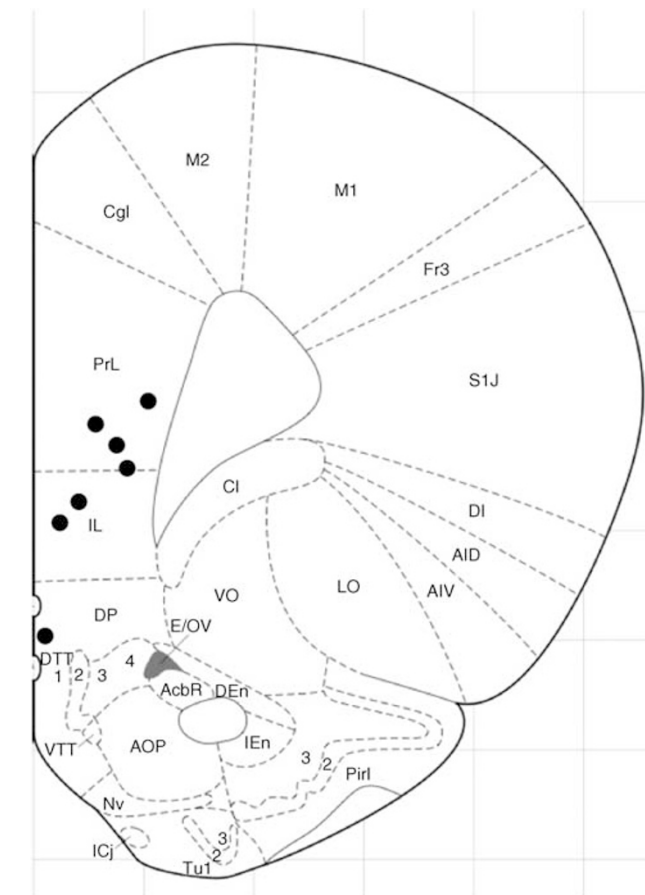
Figure 1 Coronal rat brain section of the medial PFC (AP +3.24 mm from bregma) showing actual sensor placement. Each black circle represents the tip placement of one electrode (*n* = 7). Reprinted from The Rat Brain in Stereotaxic Coordinates (Paxinos and Watson, 2005) with permission from Elsevier.

the same cage at subsequent tests. The acoustic stimuli consisted of white noise, which was delivered by two high-frequency loudspeakers built into the ceiling of the cabinet.

PPI paradigm. Each test session started with an 8-min adaptation period containing only white background noise at 62 dB(A). Startle pulse was set to 105 dB(A) and prepulse intensities to 9, 12, and 15 dB(A) above background. Duration of acoustic stimuli was set to 20 ms for both prepulses and startle pulses and interstimulus interval (ISI) was set to 40 ms. The prepulse and pulse were discrete, and separated by the ISI. After the adaptation period, the animals were subjected to a series of five startle pulse-alone trials that were omitted from the analysis because they only served to accommodate the animals to the sudden stimulus onset. The animals were then subjected to a pseudorandomized combination of 3 prepulse-alone trials for each prepulse intensity, 45 pulse-alone trials, and 15 prepulse + pulse trials for each prepulse intensity. Trials were separated by 5–15 s intervals and all these intervals included a measurement of intertrial activity. This served as a general marker of basal animal activity (not stimulus evoked) throughout the experiment. The full PPI test lasted approximately 24 min including the adaptation period.

Electrochemical Detection of Nitric Oxide

Prefrontal cortex NO levels were determined using a NO selective amperometric microsensor. The microsensor is a Nafion®-modified Pt disk electrode (patent no. S2007/00774). The sensor design has been validated for *in vitro* and *in vivo* NO sensitivity (Brown *et al*, 2005; Finnerty, 2008) and *in vitro* selectivity against ascorbic acid, uric acid, and dopamine (Brown and Lowry, 2003). The NO oxidation current (electrode potential of $+0.90$ V against an Ag reference electrode) was detected using a low-noise potentiostat (Biostat II; Electrochemical and Medical Systems, UK) and converted using an A/D converter (PowerLab; ADInstruments, United Kingdom). The digital signal was then recorded using Chart software (v5; ADInstruments) running on a PC. Each animal was connected to the *in vivo* voltammetry equipment on the day before an experiment to allow the NO oxidation current to reach a stable baseline. All experiments were carried out with the animal in its home cage. Rats were chosen for this experiment because the characterization of sensors has been carried out on this species and due to limitations regarding the size of the equipment.

Experimental Layout

All mice used for PPI testing were subjected to a pretest before surgery and/or drug treatment to ensure that they expressed basal startle reactivity.

Experiment 1: baclofen 2.5 mg/kg. Each animal ($n = 16$) received all the four treatment combinations (sal + sal, baclofen + sal, sal + PCP, and baclofen + PCP) in a balanced crossover design. Each test was separated by a 3- to 4-day long washout period. Mice were first pretreated with baclofen at a dose of 2.5 mg/kg or saline and 53 min, later they received the second injection consisting of PCP (5 mg/kg) or saline. Seven minutes after the last injection the animals were placed in the startle cages for the adaptation period and subsequent PPI test.

Experiment 2: baclofen 5 mg/kg. The mice ($n = 16$) were treated exactly as in the above experiment, but with a baclofen dose of 5 mg/kg.

Experiment 3: local injection of baclofen into the medial prefrontal cortex. At the day of the experiment a dummy cannula was inserted and retracted from the cannula guides to reduce the risk of spreading depression. After this, the animals ($n = 31$) were allowed to rest for 60 min before a first PPI test. Based on the results of this test the animals were divided into four treatment groups with comparable basal PPI and startle reactivity: veh + sal ($n = 8$); baclofen + sal ($n = 8$); veh + PCP ($n = 7$), and baclofen + PCP ($n = 9$). The mice then received a bilateral injection of either 1 µl baclofen or vehicle (Ringer's solution) -1.6 mm below the brain surface. The local injection lasted 1 min and the cannula was left in place for another 45 s to allow diffusion of the drug. Immediately after the local injection the animals were administered PCP (5 mg/kg) or saline systemically and 7 min later they were placed in the startle chambers for the second PPI test.

Experiment 4: low dose of L-NAME. As above, the experiment used a balanced crossover design with a 3- to 4-day long washout period. Each animal ($n = 16$) received all the four treatment combinations (sal + sal, L-NAME + sal, sal + PCP, and L-NAME + PCP). Pretreatment with a subthreshold dose of L-NAME (20 mg/kg) preceded the PCP injection (5 mg/kg) by 10 min. Seven minutes later, the mice were placed in the startle cages.

Experiment 5: baclofen 2.5 mg/kg in combination with low dose of L-NAME. To examine the putative interactions between GABA and NO signaling in the brain mice ($n = 16$) were pretreated with a low dose of baclofen (2.5 mg/kg) in combination with a subthreshold dose of L-NAME (20 mg/kg). Each mouse received all of the following four treatment combinations separated by a 3- to 4-day washout period: sal + sal + sal; baclofen + L-NAME + sal; sal + sal + PCP; and baclofen + L-NAME + PCP. These injections were identical in timing to the earlier systemic experiments using these compounds and thus also followed by a PCP injection 7 min before the beginning of the PPI test.

Experiment 6: NO measurements following dose response with baclofen. Each rat was subjected to four drug treatments with a 2-day washout period between each treatment. The drug treatments consisted of saline and baclofen at a dose of 2.5, 5, and 10 mg/kg (s.c.) and the doses were based on previous studies on baclofen and PPI in the rat (Bortolato *et al*, 2004). The order of the drug combinations was varied between animals in a balanced manner.

Data and Statistical Analysis

PPI. The mean response amplitude for pulse-alone trials (P) was calculated for each mouse and test. This measure was used in the statistical analysis to assess drug-induced changes in acoustic startle response. The mean response amplitude for prepulse-pulse trials (PP) was also calculated and used to express the percent prepulse inhibition according to the following formula:

$$\text{Prepulse inhibition}\,(\%) = 100 - [(PP/P) \times 100]$$

Using this formula, a 0% value denotes no difference between pulse-alone and prepulse-pulse response amplitudes and consequently no PPI. Statistical analysis for systemic experiments was performed using a three-way repeated measures ANOVA with pretreatment (baclofen, L-NAME, or sal), treatment (PCP or sal), and prepulse intensity (9, 12, and 15 dB) as within subject factors. Statistical analysis of the local injection experiments was conducted using a three-way mixed model ANOVA with pretreatment (baclofen or veh) and treatment (PCP or sal) as between-subject factors and prepulse intensity as within-subject factor. There was a significant main effect of prepulse intensity in each PPI experiment (data not shown). As no prepulse intensity × pretreatment × treatment interaction effect could be demonstrated in any of the datasets, PPI data was collapsed across prepulse intensities and presented as an average % PPI. Acoustic startle response (ASR) and intertrial activity (ITA) were analyzed using a two-way repeated measures or mixed model ANOVA with

pretreatment and treatment as within- or between-subject factors depending on experiment type. Given the *a priori* hypothesis that baclofen and L-NAME would interfere with the effects of PPI, specific differences between treatment groups were evaluated in all experiments by Bonferroni's *post hoc* tests. Two-tailed levels of significance were used and $p < 0.05$ was considered statistically significant.

NO measurements. The current over time (sampling rate 4/s) recorded in Chart was used as data. The mean of an approximately 5-min long sampling period just before drug treatment was used as baseline. The mean current change from baseline was calculated for a 5-min sampling period surrounding the time points of 30, 60, and 90 min after injection. This time frame was chosen to facilitate comparison with experiments 1–5 and previously published behavioral studies (Johansson *et al*, 1997; Klamer *et al*, 2005b; Wass *et al*, 2006a). To investigate whether the drug treatments had any significant effect on NO levels, a two-way repeated measures ANOVA with treatment and time point as within subject factors was used. In the presence of an interaction effect, his was followed by a one-way repeated measures ANOVA for each time point and where appropriate a Bonferroni's *post hoc* tests was performed for comparisons between groups. Two tailed levels of significance were used and $p < 0.05$ was considered statistically significant.

RESULTS

### Experiment 1: Baclofen 2.5 mg/kg does not Interfere with the Effects of PCP on PPI

Statistical analysis of PPI showed a significant effect of PCP (5 mg/kg) treatment (effect of treatment, $F_{(1,15)} = 25.83$, $p < 0.001$) but not of pretreatment with baclofen (2.5 mg/kg). No pretreatment $\times$ treatment interaction effect could be

demonstrated, suggesting that baclofen did not block the effects of PCP on PPI at this dose (Figure 2a). *Post hoc* comparisons showed that the baclofen + sal group did not differ from controls whereas PCP-treated animals showed a significantly lower PPI compared to the controls ($p < 0.05$). As already indicated by the lack of interaction, this effect was not prevented by pretreatment with baclofen because the PPI of the baclofen + PCP group was significantly lower than that of the control group ($p < 0.05$) but not different from the veh + PCP group.

There was no effect of drug treatment on ASR in experiment 1 (Table 1). Basal activity levels, as measured by ITA (Figure 3a), were significantly increased in PCP-treated animals (effect of treatment, $F_{(1,15)} = 47.77$, $p < 0.001$) and decreased in baclofen-treated animals (effect of pretreatment, $F_{(1,15)} = 5.505$, $p < 0.05$). Pretreatment with baclofen partially blocked the PCP-induced hyperactivity as evidenced by a significant pretreatment $\times$ treatment interaction (interaction effect, $F_{(1,15)} = 5.985$, $p < 0.05$). This was further supported by *post hoc* comparisons showing higher ITA in PCP-treated animals compared to controls ($p < 0.001$) whereas this could not be demonstrated for the baclofen + PCP group. However, the baclofen + PCP group did not show a significantly lower ITA than the PCP group, suggesting that the blockade was only partial.

Experiment 2: Baclofen 5 mg/kg Elevates Basal PPI and Prevents the Effects of PCP

PCP (5 mg/kg) had a main disruptive effect on PPI (effect of treatment, $F_{(1,15)} = 74.02$, $p < 0.001$) whereas baclofen (5 mg/kg) increased PPI significantly (effect of pretreatment, $F_{(1,15)} = 45.01$, $p < 0.001$; Figure 2b). Similar to the lower dose of baclofen, no pretreatment $\times$ treatment interaction effect could be observed, suggesting additive effects of baclofen and PCP. *Post hoc* comparisons revealed an increased PPI in the baclofen + sal group compared to
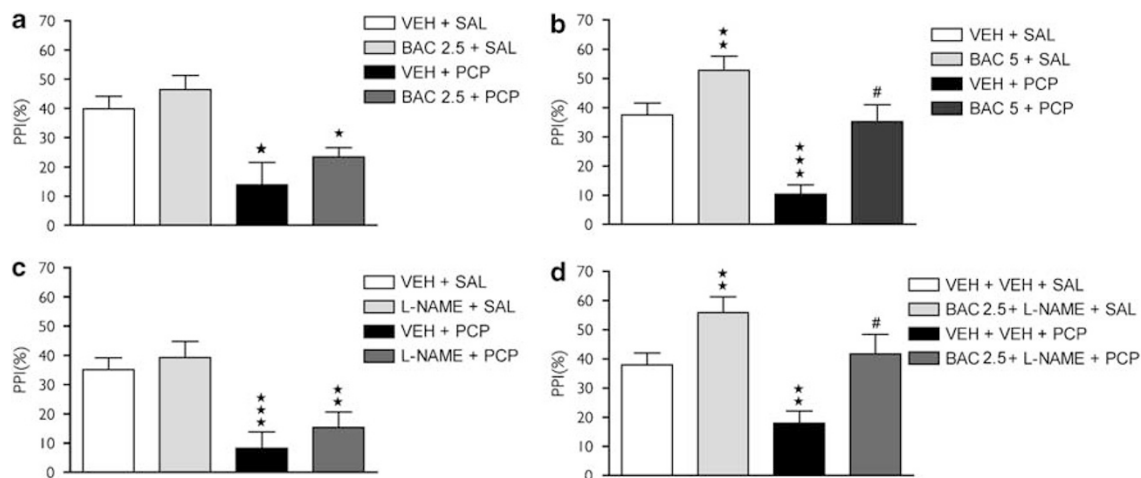
Figure 2 The effects of pretreatment with baclofen (BAC) and/or N^G-nitro-L-arginine methyl ester (L-NAME) on PCP-induced disruption of prepulse inhibition (PPI) in mice. Data are presented as mean PPI values collapsed over prepulse intensities ± SEM for each treatment group. (★$p < 0.05$, ★★$p < 0.01$, ★★★$p < 0.001$ *vs* control group; #$p < 0.05$ *vs* PCP group; Bonferroni's test). (a) A low dose of BAC (2.5 mg/kg) does not interfere with the effects of PCP (5 mg/kg) on PPI. (b) A high dose of BAC (5 mg/kg) attenuates the effects of PCP in an additive manner. (c) Pretreatment with a subthreshold dose of L-NAME (20 mg/kg) does not interfere with the effects of PCP on PPI. (d) Combination of BAC (2.5 mg/kg) and L-NAME (20 mg/kg) increases basal PPI and attenuates the effects of PCP on PPI in an additive manner.

Table 1 Acoustic Startle Response (ASR) After Different Treatment Combinations in Experiments 1–5

	Exp 1		Exp 2		Exp 3		Exp 4		Exp 5	
	VEH	**BAC 2.5**	**VEH**	**BAC 5[a]**	**VEH**	**BAC 1mM**	**VEH**	**L-NAME**	**VEH**	**BAC+L-NAME**
SAL	908 ± 104	990 ± 98.4	867 ± 96.6	501 ± 91.4[b]	505 ± 155	801 ± 202	812 ± 120	729 ± 108	1355 ± 134	1141 ± 147
PCP	821 ± 90.7	847 ± 85.2	791 ± 89.7	420 ± 62.2[b]	359 ± 92.5	430 ± 120	673 ± 68.7	686 ± 89.7	1190 ± 95.0	1101 ± 128

[a]$p < 0.05$ main effect of pretreatment following 3-way ANOVA.
[b]$p < 0.001$ *vs* control group, Bonferroni's test.
Data are presented as mean ASR values ± SEM for each treatment group. Baclofen (BAC; 2.5–5 mg/kg systemically or as bilateral microinjections 1 mM), N^G-nitro-L-arginine methyl ester (L-NAME; 20 mg/kg); and PCP (5 mg/kg) were administered in five separate experiments. The high dose of baclofen decreased ASR in experiment 2.
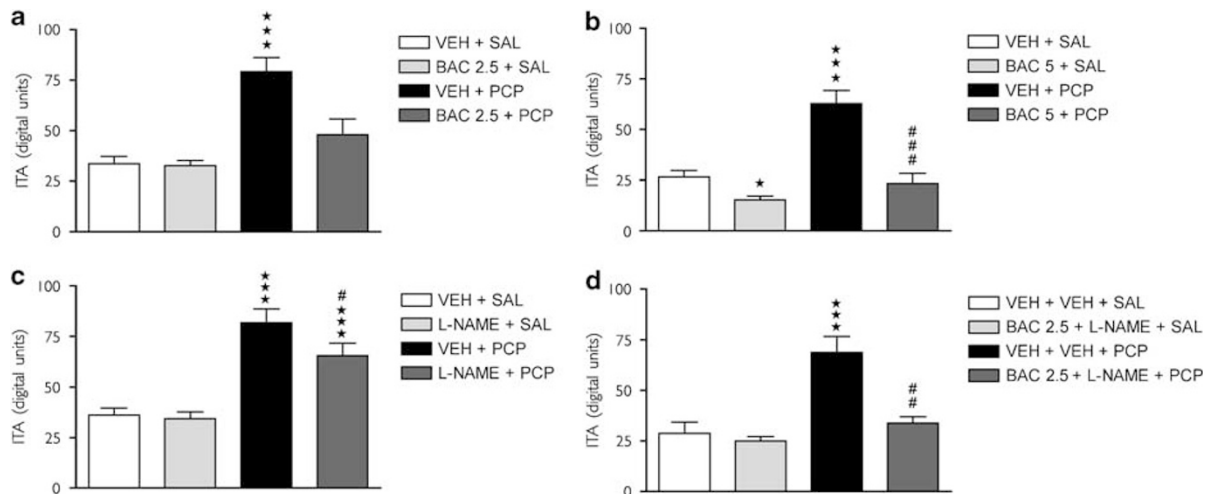
Figure 3 The effects of pretreatment with baclofen (BAC) and/or N^G-nitro-L-arginine methyl ester (L-NAME) on PCP-induced hyperactivity in mice as measured by intertrial activity (ITA). Data are presented as mean ITA values ± SEM for each treatment group. (★$p < 0.05$, ★★★$p < 0.001$ *vs* control group; [#]$p < 0.05$, [##]$p < 0.01$, [###]$p < 0.01$ *vs* PCP group; Bonferroni's test). (a) A low dose of BAC (2.5 mg/kg) partially interferes with the effects of PCP (5 mg/kg) on ITA. (b) A high dose of BAC (5 mg/kg) decreases ITA and blocks the PCP-induced hyperlocomotion. (c) Pretreatment with a subthreshold dose of L-NAME (20 mg/kg) partially attenuates the effects of PCP on ITA. (d) Combination of BAC (2.5 mg/kg) and L-NAME (20 mg/kg) blocks the PCP-induced hyperactivity.

controls ($p < 0.01$), suggesting an increasing effect of this dose of baclofen on PPI *per se*. PCP-treated animals had a lower PPI compared to controls ($p < 0.001$) and had also significantly lower PPI than the baclofen + PCP group ($p < 0.01$). Furthermore, baclofen + PCP-treated animals did not differ significantly from controls, indicating a normalization of PCP-induced PPI deficits by pretreatment with baclofen.

Statistical analysis of ASR (Table 1) showed that baclofen significantly lowered this measure (effect of pretreatment, $F_{(1,15)} = 37.80$, $p < 0.001$) whereas no main effect of PCP treatment could be demonstrated. *Post hoc* comparisons showed that both the baclofen + sal group and the baclofen + PCP group had significantly lower ASR than controls ($p < 0.001$ for both comparisons). Analysis of ITA showed a similar pattern to experiment 1 (Figure 3b). ITA was increased by PCP (effect of treatment, $F_{(1,15)} = 29.57$, $p < 0.001$), decreased by baclofen (effect of pretreatment, $F_{(1,15)} = 31.44$, $p < 0.001$), and a significant pretreatment × treatment interaction could be demonstrated (interaction effect, $F_{(1,15)} = 23.18$, $p < 0.001$). *Post hoc* comparisons showed a lower ITA in baclofen-treated mice compared to

controls ($p < 0.05$). PCP-treated animals had a higher ITA than both controls ($p < 0.001$) and baclofen + PCP-treated animals ($p < 0.001$) suggesting a normalization of activity levels by baclofen. This was further supported by an absence of difference between the baclofen + PCP group and controls.

Experiment 3: Prefrontal Injection of Baclofen Blocks PCP-Induced PPI Deficits

Systemic PCP (5 mg/kg) injection disrupted PPI (effect of treatment, $F_{(1,28)} = 8.744$, $p < 0.01$) whereas no main effect of local pretreatment with baclofen (1 mM) could be shown (Figure 4a). A significant pretreatment × treatment interaction effect indicated that prefrontal pretreatment with baclofen blocked the PPI deficits induced by systemic PCP administration (interaction effect, $F_{(1,28)} = 5.597$, $p < 0.05$). *Post hoc* analysis further supported this interpretation as PCP-treated animals had lower PPI than both controls ($p < 0.01$) and baclofen + PCP-treated animals ($p < 0.05$). In addition, the PPI of the baclofen + PCP group did not differ
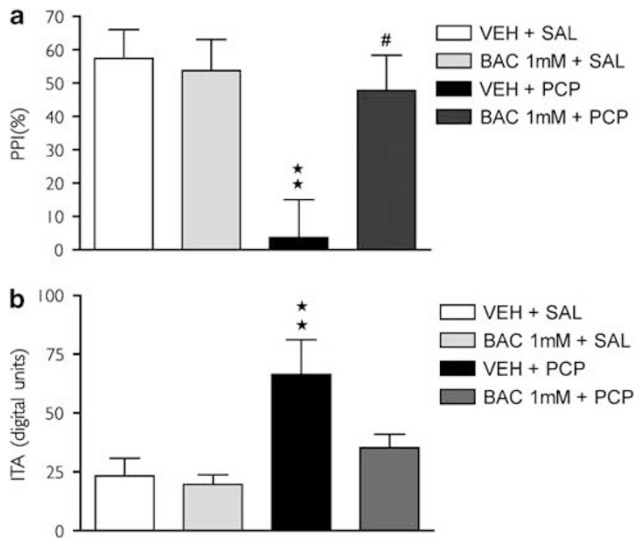
Figure 4 The effects of bilateral baclofen treatment (BAC; 1 mM) into the medial PFC followed by systemic PCP administration (5 mg/kg) on prepulse inhibition (PPI) and intertrial activity (ITA). Data are presented as mean PPI values collapsed over prepulse intensities ± SEM for each treatment group. (★★ $p < 0.01$ vs control group; # $p < 0.05$ vs PCP group; Bonferroni's test). (a) Prefrontal pretreatment with BAC blocks PCP-induced PPI disruption. (b) Prefrontal pretreatment with BAC partly blocks PCP-induced hyperactivity.

from the control group, suggesting a full blockade of the effects of PCP.

ASR was not affected by any treatment combination in experiment 3 (Table 1). ITA levels were significantly increased in PCP-treated animals (effect of treatment, $F_{(1,28)} = 12.18$, $p < 0.01$) and animals locally pretreated with baclofen showed a decreased ITA (effect of pretreatment, $F_{(1,28)} = 4.234$, $p < 0.05$; Figure 4b). No pretreatment × treatment interaction effect was present. *Post hoc* comparisons revealed a significantly increased ITA in the PCP group ($p < 0.01$) compared with controls, but did not show a difference between the baclofen + sal group and controls. Furthermore, PCP-treated animals did not differ from the baclofen + PCP group suggesting an inability of locally administered baclofen to block PCP-induced hyperactivity. However, the ITA of the baclofen + PCP group was not different from that of the control group, which in the absence of an interaction effect can be interpreted as a tendency to attenuate the effects of PCP.

Experiment 4: Pretreatment with a Low Dose of L-NAME does not Interfere with the Effects of PCP

L-NAME (20 mg/kg) administration did not produce a main effect on PPI whereas PCP significantly decreased PPI (effect of treatment, $F_{(1,15)} = 40.15$, $p < 0.001$; Figure 2c). No pretreatment × treatment interaction effect could be demonstrated and *post hoc* comparisons did not show any significant differences between the L-NAME + PCP group and PCP-treated animals. Both PCP-treated animals and the L-NAME + PCP group differed significantly from controls, confirming that this dose of L-NAME did not attenuate the effects of PCP on PPI.

ASR was not affected by any treatment combination in experiment 4 (Table 1). Statistical analysis showed a significant increase in ITA levels following PCP treatment (effect of treatment, $F_{(1,15)} = 37.94$, $p < 0.001$; Figure 3c) and a significant effect following pretreatment with L-NAME (effect of pretreatment, $F_{(1,15)} = 12.18$, $p < 0.001$). Furthermore, a significant pretreatment × treatment interaction effect for ITA could be demonstrated (interaction effect, $F_{(1,15)} = 6.220$, $p < 0.05$). *Post hoc* comparisons of ITA levels showed that PCP-treated animals had significantly higher ITA levels than controls ($p < 0.05$). L-NAME + PCP-treated animals had lower ITA levels than PCP-treated animals ($p < 0.05$), but also showed a significant increase in ITA compared to controls ($p < 0.001$) suggesting a partial attenuation of the PCP-induced hyperactivity.

Experiment 5: Baclofen + L-NAME Combination Increases Basal PPI and Prevents the Effects of PCP

Pretreatment with subthreshold doses of baclofen (2.5 mg/kg) and L-NAME (20 mg/kg) significantly increased PPI (effect of pretreatment, $F_{(1,15)} = 21.58$, $p < 0.001$; Figure 2d). As above, PCP-treatment decreased PPI levels (effect of treatment, $F_{(1,15)} = 31.41$, $p < 0.001$) but no pretreatment × treatment interaction was present, suggesting additive effects of these two factors on PPI. *Post hoc* comparisons confirmed that the combined pretreatment increased PPI in an additive manner as the baclofen + L-NAME + sal group had significantly higher PPI than controls ($p < 0.01$). Additionally, the pretreatment appeared to prevent the effects of PCP, as the baclofen + L-NAME + PCP group had significantly higher PPI than PCP-treated animals ($p < 0.05$) but did not differ from controls.

ASR was not affected by any treatment combination in experiment 5 (Table 1). ITA was significantly increased after PCP treatment (effect of treatment, $F_{(1,15)} = 29.26$, $p < 0.001$) and lowered after pretreatment with baclofen and L-NAME (effect of pretreatment, $F_{(1,15)} = 12.22$, $p < 0.01$; Figure 3d). A significant pretreatment × treatment interaction (interaction effect, $F_{(1,15)} = 16.66$, $p < 0.001$) suggested that the combined pretreatment blocked the effects of PCP on ITA. *Post hoc* analysis confirmed this as the significant increase in ITA levels following PCP ($p < 0.001$) could not be seen for the baclofen + L-NAME + PCP group whereas the latter group had significantly lower ITA than PCP-treated animals ($p < 0.01$).

Experiment 6: Baclofen Decreases NO Levels in the mPFC

Baclofen treatment significantly decreased the NO current over the sampling time (effect of treatment, $F_{(3,18)} = 4.233$, $p < 0.05$; Figure 5). Furthermore, this effect was time dependent as evidenced by a treatment × time point (30, 60, or 90 min) interaction effect (interaction effect, $F_{(6,36)} = 2.772$, $p < 0.05$). This warranted a detailed analysis for each time point. Analysis of the change in NO current at 30 min revealed no significant effect of baclofen treatment. At 60 min after injection, a main effect of baclofen could be detected (effect of treatment, $F_{(3,18)} = 3.264$, $p < 0.05$) suggesting a decrease in NO current due to baclofen treatment. However, *post hoc* comparisons did not reveal
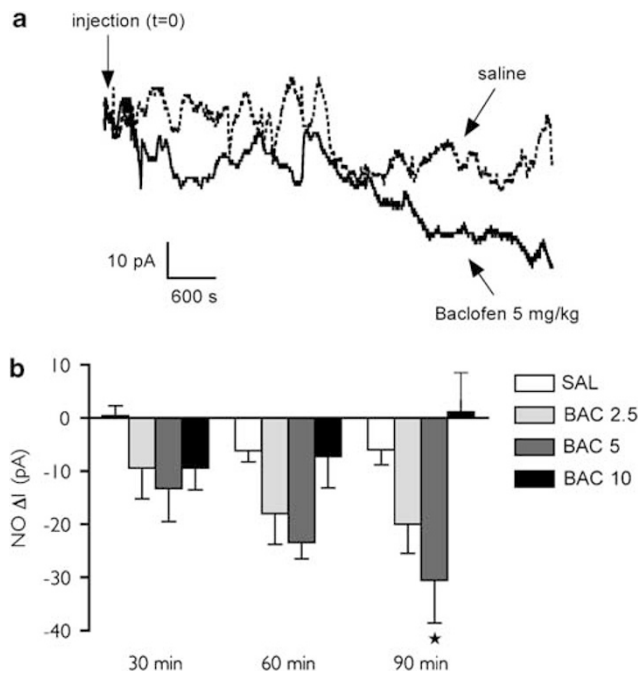
Figure 5 Prefrontal NO release is decreased following systemic baclofen administration. (a) A representative voltammogram showing the oxidation current in the medial prefrontal cortex after saline or baclofen (5 mg/kg, $t=0$) administration. Data are expressed as current (pA) over time (seconds). (b) Change in prefrontal NO release 30, 60, and 90 min after systemic baclofen administration. Data are presented as mean change in NO current compared to baseline ± SEM for each treatment group (★$p<0.05$ vs control group, Bonferroni's test).

any significant difference between the control group and the different treatment groups, indicating that this decrease was relatively subtle. Finally, analysis of the change in NO current 90 min after injection revealed a significant decrease following baclofen treatment (effect of treatment, $F_{(3,18)}=5.118$, $p<0.01$). *Post hoc* comparisons showed that baclofen 5 mg/kg ($p<0.05$) significantly decreased NO levels, whereas this was not the case for 2.5 mg/kg or the 10 mg/kg dose. This indicates a dose-related effect of baclofen on NO levels in the medial PFC.

DISCUSSION

In this study, PCP-induced PPI deficits and hyperactivity were attenuated by systemic pretreatment with the GABA$_B$ agonist baclofen in a dose-related manner. Although systemic baclofen normalized PPI in an additive manner, prefrontal microinjections of baclofen completely blocked the PPI-disruption caused by PCP, without affecting this measure *per se*. Furthermore, the combination of baclofen and an NOS inhibitor was more effective in preventing the effects of PCP than any compound by itself. Additionally, *in vivo* measurements of NO levels showed that baclofen inhibited NO release in the PFC.

Effects of Baclofen

The finding that baclofen attenuates the disruptive effects of PCP on PPI, extends previous studies showing that systemic administration of this compound blocks the effects of the

NMDA receptor antagonist, dizocilpine (MK-801), in both rats (Bortolato *et al*, 2004) and mice (Arai *et al*, 2008). In addition, baclofen improves spontaneous PPI deficits in DBA/2J mice without affecting basal PPI in control mice (Bortolato *et al*, 2007). The above-mentioned studies have all demonstrated effects in a dose range similar to this study, although direct comparisons are somewhat complicated by interstudy differences in mice strain, rodent species, or baclofen enantiomer. One important difference is that baclofen (5 mg/kg) increased basal PPI in this study and thus attenuated the effects of PCP in an additive rather than interactive manner. Although such an increase is at variance with previous studies, the present findings point to a role of GABA$_B$ receptors in the regulation of preattentive information processing, possibly indicating that stimulation of these receptors may have an antipsychotic effect in patients. Early studies of the antipsychotic potential of baclofen showed beneficial effects in patients with schizophrenia (Frederiksen, 1975; Schopf and Hucker, 1977), although these findings were not replicated in later studies (Beckmann *et al*, 1977; Bigelow *et al*, 1977; Gulmann *et al*, 1976). In this study, the higher dose of baclofen (5 mg/kg) significantly lowered startle reactivity, possibly due to the muscle-relaxing properties of this compound. However, PPI does not appear to be correlated to either drug-induced or genotype-induced differences in ASR, suggesting that the decrease in ASR was not likely confounding the PPI measurements in experiment 2 (Klamer *et al*, 2005b; Paylor and Crawley, 1997).

At the lower dose (2.5 mg/kg) baclofen also blocked the hyperactivity caused by PCP as measured by ITA without affecting this measure in itself, in analogy to an earlier study in mice (Menon *et al*, 1980). A possible mechanism behind this may be that baclofen stabilizes dopamine neuron firing in the midbrain (Erhardt *et al*, 1998, 2002). In support of this, baclofen has proven effective against the disruption of PPI by metamphetamine (Arai *et al*, 2008), D-amphetamine-induced hypermotility and disruption of successive discrimination (Ahlenius *et al*, 1975; Phillis *et al*, 2001; Zhou *et al*, 2004). Although the hyperactivity caused by common psychotomimetics such as D-amphetamine, traditionally has been attributed to stimulation of the mesocorticolimbic dopamine system, this may not be true for NMDA receptor antagonists such as PCP where the effects on locomotion are temporally dissociated from changes in dopamine levels (Adams and Moghaddam, 1998). The present finding that prefrontal injections of baclofen partially block PCP-induced hyperactivity may be an additional sign that also changes in nondopaminergic cortical transmission should be considered as a part of the explanation.

Regional Effects of Baclofen

Local injection of baclofen in the medial PFC effectively blocked the PPI disruption caused by systemic PCP administration without affecting this measure *per se*. The apparent difference in the effects of systemic and prefrontal administration of baclofen on baseline PPI may result from the interference with GABA signaling at multiple brain regions following systemic injection. PPI is known to be modulated in a top-down manner by the PFC, and also by brain nuclei more proximal to the primary startle circuit

such as the lateral globus pallidus (LGP) and the pedunculopontine nucleus (PPTg; Koch and Schnitzler, 1997; Kodsi and Swerdlow, 1997; Swerdlow et al, 2001). Interestingly, it was recently shown that PPTg-targeting GABAergic neurons in the LGP are activated at both prepulse and prepulse + pulse (PPI) conditions, and that transient inactivation of this region by lidocaine decreases PPI in mice. Furthermore, infusions of both GABA$_A$ and GABA$_B$ receptor antagonists into the PPTg decrease PPI, suggesting that the LGP regulates PPI by acting on GABA receptors in the PPTg (Diederich and Koch, 2005; Takahashi et al, 2007). Thus, it is possible that systemic (but not prefrontal) injections of baclofen used in this study, affects PPTg in the opposite direction, thereby causing an increase in basal PPI.

The present findings suggests that impairments in prefrontal GABA$_B$-mediated inhibition may be important for the effects of PCP on information processing and that restoration of inhibitory signaling through these receptors is sufficient to normalize PPI. It is now known that NMDA receptor antagonists stimulate cortical glutamate and dopamine release (Adams and Moghaddam, 1998; Moghaddam et al, 1997) and suppress inhibitory synaptic transmission (Zhang et al, 2008). Interestingly, perfusion of baclofen into the PFC has been demonstrated to decrease both basal dopamine release and NO release in this region (Ishizuka et al, 2000; Santiago et al, 1993) and also to block the behavioral arousal and hyperlocomotion caused by the NMDA receptor antagonist AP-5 (Feenstra et al, 2002). Prefrontal injections of baclofen also inhibit stimulated glutamate release in the ventral tegmental area, which receives corticofugal projections from the PFC and consequently reflects the degree of prefrontal excitation (Harte and O'Connor, 2005). Thus, the present effects of baclofen may be explained as a restoration of inhibitory function followed by stabilization of aberrant glutamate release and/or by an interaction with the dopamine and NO systems in the PFC. Notably, GABA$_B$ receptor expression appears to be reduced both in the PFC, entorhinal cortex, and hippocampus of subjects with schizophrenia, indicating that these receptors may have an important role in the pathophysiology of the disease (Ishikawa et al, 2005; Mizukami et al, 2000, 2002).

GABA$_B$ and Nitric Oxide

The combination of subthreshold doses of baclofen and L-NAME was more effective in preventing the effects of PCP than any compound by itself. However, the increasing effect of pretreatment on PPI, in combination with the absence of a pretreatment × treatment interaction, suggests that this effect mainly is of an additive nature. An important observation is that neither baclofen nor L-NAME (at subthreshold doses) increased basal PPI levels, whereas this effect was evident for their combination. This finding opens up the possibility of a connection between GABA$_B$ receptors and NO release in the regulation of PPI. A similar relationship between these two signaling systems has previously been described in the hippocampus where basal cGMP efflux is increased by local administration of GABA$_B$ antagonists and decreased by baclofen (Fedele et al, 1997). Future studies using local pretreatment with GABA$_B$

antagonists in the PFC, may clarify whether the observed normalizing effect of baclofen/L-NAME on disrupted PPI is dependent on prefrontal GABA$_B$ receptor signaling.

In this study, systemic baclofen administration decreased NO levels over time in the rat medial PFC, indicating that cortical NO release may be under the influence of upstream GABA$_B$ receptor signaling. It has been shown that release of both glutamate and the effector of NO signaling, cGMP, are decreased by baclofen but increased by GABA$_B$ antagonists (Fedele et al, 1997; Harte and O'Connor, 2005; Waldmeier et al, 2008). Consequently, antagonists of the GABA$_B$ receptor may elevate NO levels, possibly through a disinhibitory activation of glutamate receptors. A similar disinhibition pattern could theoretically arise from blocking NMDA receptors on interneurons with PCP, where an increase in glutamate, instead, may act on Ca^{2+}-permeable AMPA receptors to regulate NO release. Interestingly most neurons that express this subtype of AMPA receptor and/or produce NO are GABAergic interneurons (Gabbott and Bacon, 1995; Jonas et al, 1994; Szabadits et al, 2007; Yan et al, 1996; Yin et al, 1994).

The decrease in NO levels was most pronounced at a time point (60–90 min) coinciding with the time for PPI measurements following baclofen injection. Tentatively, the temporal association between the effects of baclofen on PPI and NO release gives further support to the hypothesis that NO signaling in the medial PFC may be partly responsible for the effects of baclofen on PPI. Although the medium dose of baclofen (5 mg/kg) potently decreased NO levels, this effect was completely absent for the highest dose (10 mg/kg). This 'inverted U'-like shape of the dose response curve remains to be explained, but may possibly reflect a more widespread engagement of GABA$_B$ receptors in the brain, paralleled with the visibly decreased basal activity at this dose, which is likely to be caused by the muscle-relaxing and sedative effects of baclofen (Nevins et al, 1993). Additionally, GABA$_B$ receptors are coupled to G proteins, Ca^{2+} channels, K^+ channels, and adenylyl cyclase, and are situated both presynaptically as auto- or heteroreceptors and at postsynaptic neurons. Thus, these receptors are capable of modulating release of various neurotransmitters as well as hyperpolarizing the postsynaptic membrane potential (for review see Bettler et al, 2004; Couve et al, 2000) and this complexity should call for caution when interpreting the above-mentioned results.

Concluding Remarks

This study demonstrates that signaling through GABA$_B$ receptors mediates certain effects of PCP on information processing and that the PFC is strongly implicated in these effects. Furthermore, an interaction between GABA$_B$ and NO signaling was detected as evidenced by converging effects of these pathways on PPI and on prefrontal NO release in vivo. Baclofen had an effect on PPI per se following systemic, but not prefrontal injections, tentatively indicating that GABA$_B$ receptors in the PFC are involved in the effects of PCP but not in the modulation of baseline PPI. Taken together, the present findings support recent hypotheses on compromised inhibition following administration of NMDA receptor antagonists leading to a disinhibition of the PFC (Tanaka, 2008; Zhang et al,

2008). Interestingly, activation of the hippocampus and PFC in patients with schizophrenia has been demonstrated in some, but not all, studies of cerebral blood flow (Catafau et al, 1994; Friston et al, 1992; Ingvar and Franzen, 1974; Malaspina et al, 2004; Taylor et al, 2007). The recent finding that the mGlu2/3 receptor agonist LY2140023 significantly improves positive and negative symptoms in schizophrenia (Patil et al, 2007), also points in the direction of disinhibition, as the most likely effect of this compound is to reduce glutamate signaling in the brain. This study proposes a role for GABA_B receptor signaling in the effects of PCP, possibly with altered NO levels as a downstream mediator. These results expand the recent finding that prefrontal NO/sGC signaling is important for the effects of PCP (Fejgin et al, 2008) and provide behavioral and biochemical support for NO as an effector of a disinhibited PFC. Future studies of the glutamate/GABA_B/NO signaling pathway may bring greater insight into the mechanisms underlying cortical disinhibition in relation to disrupted information processing, and thus their putative importance for cognitive deficits in schizophrenia.

DISCLOSURE/CONFLICTS OF INTEREST

The authors hereby declare that no financial support or compensation has been received from any individual or corporate entity over the past 3 years for research or professional service, and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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