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ORIGINAL INVESTIGATION

Information processing deficits and nitric oxide signalling in the phencyclidine model of schizophrenia

Erik Pålsson · John Lowry · Daniel Klamer

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Abstract
Rationale Schizophrenia-like cognitive deficits induced by phencyclidine (PCP), a drug commonly used to model schizophrenia in experimental animals, are attenuated by nitric oxide (NO) synthase inhibitors. Furthermore, PCP increases NO levels and sGC/cGMP signalling in the prefrontal cortex in rodents. Hence, a cortical NO/sGC/cGMP signalling pathway may constitute a target for novel pharmacological therapies in schizophrenia.
Objectives The objective of this study was to further investigate the role of NO signalling for a PCP-induced deficit in pre-attentive information processing.
Materials and methods Male Sprague Dawley rats were surgically implanted with NO-selective amperometric microsensors aimed at the prefrontal cortex, ventral hippocampus or nucleus accumbens, and NO levels and prepulse inhibition (PPI) were simultaneously assessed.
Results PCP treatment increased NO levels in the prefrontal cortex and ventral hippocampus, but not in the nucleus accumbens. The increase in NO levels was not temporally correlated to the deficit in PPI induced by PCP. Furthermore, pretreatment with the neuronal NO synthase inhibitor *N*-propyl-L-arginine dose-dependently attenuated both the increase in prefrontal cortex NO levels and the deficit in PPI.
Conclusions These findings support a demonstrated role of NO in the behavioural and neurochemical effects of PCP. Furthermore, this effect is brain region-specific and mainly involves the neuronal isoform of NOS. However, a temporal correlation between a PCP-induced disruption of PPI and an increase in prefrontal cortex NO levels was not demonstrated, suggesting that the interaction between PCP and the NO system is more complex than previously thought.

Keywords Prepulse inhibition · Rat · Nitric oxide · Schizophrenia · Voltammetry · Phencyclidine · Prefrontal cortex · Nitric oxide synthase · NMDA receptor

E. Pålsson (✉) · D. Klamer
Department of Pharmacology, Institute of Neuroscience and Physiology, Sahlgrenska Academy at University of Gothenburg, POB 431, 405 30 Gothenburg, Sweden
e-mail: erik.palsson@pharm.gu.se

E. Pålsson · J. Lowry
Department of Chemistry,
National University of Ireland Maynooth,
Maynooth, Ireland

Introduction

Aberrations in nitric oxide (NO) signalling have been associated with schizophrenia in both clinical studies and animal models of the disorder (Baba et al. 2004; Bernstein et al. 2001; Bird et al. 2001). Furthermore, genetic studies support a role of polymorphisms in neuronal NO synthase (NOS) gene as a risk factor for schizophrenia (Reif et al. 2006; Wratten et al. 2009). In addition, a number of studies in rodents using phencyclidine (PCP), a drug commonly used to model schizophrenia in experimental animals, show a beneficial effect of NOS inhibitors on schizophrenia-related cognitive deficits (Johansson et al. 1997; Klamer et al. 2004d, 2005b; Wass et al. 2006, 2009). Such an effect has also been demonstrated using neuronal NOS-selective inhibitors, suggesting that the NO-dependent effects of PCP involve increased neuronal NO production (Klamer et al. 2004a; Wiley 1998). A role for NO dysregulation is supported by recent findings showing increases in NO levels and NO-dependent increases in cGMP signalling in the prefrontal cortex following PCP administration in rodents (Fejgin et al. 2008). Furthermore, blocking the

production of cGMP in the prefrontal cortex prevented a PCP-induced deficit in pre-attentive information processing (Fejgin et al. 2008). Taken together, these observations suggest that at least some of the schizophrenia-related behavioural effects of PCP are caused by the activation of the prefrontal cortex neuronal NOS and the subsequent activation of the cGMP signalling cascade. It should be noted that there are discordant reports demonstrating that the NO donor sodium nitroprusside can attenuate PCP-induced behavioural effects (Bujas-Bobanovic et al. 2000). Thus, the relationship between NO and the schizophrenia-like effects of PCP is still unclear. However, the prefrontal cortex NO/sGC/cGMP signalling pathway may constitute an interesting target for novel pharmacological therapies in schizophrenia and possibly play a role in the pathophysiology of the disorder. In support of this, the NOS and sGC inhibitor methylene blue has been shown to have some effect as an adjuvant treatment in schizophrenia patients (Deutsch et al. 1997) and to attenuate the effects of PCP in rodents (Klamer et al. 2004b). Again, there are conflicting data as the phosphodiesterase inhibitor sildenafil failed to improve cognitive function in a placebo-controlled study despite beneficial effects in animal models (Goff et al. 2009).

In previous studies, we have used separate experiments to study the biochemical and behavioural NO dependence of PCP in rodents. Thus, a direct causal link between the observed increases in prefrontal cortex NO signalling and schizophrenia-like behavioural effects remains to be shown. In the present study, the role of NO signalling in the behavioural and biochemical effects of PCP was further investigated using simultaneous electrochemical detection of NO levels and assessment of pre-attentive information processing using prepulse inhibition (PPI) following PCP administration. Prepulse inhibition of the acoustic startle reflex is a translational behavioural paradigm that is widely used to assess pre-attentive information processing in schizophrenia models. Deficits in PPI are present in schizophrenia patients and are also found in experimental animals following administration of non-competitive NMDA receptor antagonists (Braff et al. 2001; Swerdlow et al. 2008). In the first series of experiments, rats were systemically treated with PCP and tested for PPI with concomitant measurement in NO levels in the prefrontal cortex, ventral hippocampus and nucleus accumbens. These brain regions were chosen based on their suggested involvement in both the pathophysiology of schizophrenia and the modulation of the PPI response. In a second series of experiments, rats were systemically treated with the neuronal NOS-selective inhibitor *N*-propyl-L-arginine (LNPA) and PCP and tested for PPI with concomitant measurement of NO levels in the prefrontal cortex to further investigate the involvement of the neuronal NOS isoform in a PCP-induced deficit in PPI.

Materials and methods

Animals

Male Sprague Dawley rats (Taconic, Denmark, 280 400 g) were used. All animals were housed, with a maximum of four per cage (55×35×20 cm), in a colony room under constant temperature (20±1°C) and humidity (50±5%). Food and water were available ad libitum. The daylight cycle was maintained artificially (lights on from 0600 to 1800 hours), and the experiments were conducted during the light phase. The animals were allowed to acclimatise for at least 1 week prior to surgery. All experimental procedures used in the present study were approved by the Ethics Committee for Animal Experiments, Goteborg.

Drugs

Phencyclidine hydrochloride (PCP, Sigma Chemicals, St. Louis, MO, USA) and LNPA (Tocris, Bristol, UK) were used in the present study. PCP and LNPA were dissolved in saline (0.9% NaCl) and injected subcutaneously (s.c.) in a volume of 2 ml/kg. The doses of PCP and LNPA used were based on previously published studies (Johansson et al. 1997; Klamer et al. 2001, 2004a).

Surgical procedure

The rats were anaesthetised 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. Two holes for the anchor screws, two holes for the reference (8-T Ag wire, 200-μm bare diameter; Advent Research Materials, UK) and auxiliary (8-T Ag wire) electrodes and one hole for the working (sensor) electrode were drilled. Electrodes were then implanted following a previously described procedure (Lowry et al. 1997). The coordinates used for the medial prefrontal cortex 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. Similarly, the coordinates for the ventral hippocampus were 5.2, ±5.4 and 6.0 mm and for the nucleus accumbens +1.85, ±1.3 and 6.8 mm. The electrodes were inserted into the brain and connected to a pedestal that was secured to the anchor screws with dental cement. During surgery, the rats were administered 2.0 ml of saline to reduce postoperative dehydration and an analgesic (carprofen) to reduce postoperative pain. The animals were allowed to recover for 2 3 days before commencing experiments. They were housed individually in standard plastic cages (35×20×16 cm).

Electrochemical detection of NO

Brain NO levels were determined using a NO-selective amperometric microsensor. The microsensor is a Nafion-modified Pt disk electrode (patent no. S2007/00774, Blue Box Sensors Ltd., Dublin, Ireland). The sensor design has been validated for in vitro and in vivo NO sensitivity (Brown et al. 2005, 2009) 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, UK). The digital signal was then recorded using Chart software (v5, ADInstruments) running on a PC. Individual working (sensor) electrodes were tested for ascorbic acid interference and calibrated to ensure NO sensitivity in vitro prior to surgery.

Prepulse inhibition experiments

Apparatus

Acoustic startle was recorded by a MOPS 3 startle response recording system (Metod och Produkt Svenska AB, Sweden). The animals were placed in a circular cabinet (diameter, 40 cm; wall height, 24 cm). A plastic horizontal disk (diameter, 39.5 cm) attached on top of the moving coil transducer was used as floor in the arena. The rat movements caused a displacement of the disk, the acceleration of which was converted to an analogue signal by the transducer. This signal was sampled and digitised 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. The acoustic stimuli consisted of white noise, which was delivered by three high-frequency loudspeakers built into the ceiling of the cabinet.

PPI paradigm

Each test session started with a 10-min adaptation period containing only white background noise at 62 dB(A). Startle pulse was set to 105 dB(A) and prepulse intensity to 15 dB(A) above background. Duration of acoustic stimuli was set to 20 ms for both prepulses and startle pulses, and interstimulus interval was set to 40 ms. Following the adaptation period, the animals were subjected to a pseudo-randomised combination of three prepulse-alone trials, 15 pulse-alone trials and 15 prepulse+pulse trials. Trials were separated by 5- to 15-s intervals. The duration of the PPI test was approximately 4 min, and the test was repeated for a total of 12 times with 10-min background noise only intervals between each test. The total length of the test session was approximately 168 min.

Experimental design

Each rat was connected to the in vivo voltammetry equipment on the day before the experiment to allow the NO oxidation current to reach a stable baseline. Every animal was initially administered saline (2 ml/kg, s.c.) and placed in the circular arena. After 5 min, they were exposed to white background noise only for 180 min to allow the animals to acclimatise to the test procedure. After a 2-day washout period, two PPI test sessions were conducted. All animals received both treatment combinations in a balanced crossover design. Each test was separated by a 3- to 4-day-long washout period. In experiment 1, each rat received an injection of saline or PCP (2 mg/kg) and was then placed in the arena, and 5 min later, the PPI test was started. In experiment 2, rats were first pretreated with LNPA (5 or 10 mg/kg, s.c.) and 10 min later received a second injection of saline or PCP (2 mg/kg). The animal was then placed in the arena, and 5 min later, the PPI test was started.

Probe placement verification

After termination of the experiments, the rats were decapitated. The brains were removed and frozen at 80°C. Sensor placement was verified by sectioning the brains with an atlas of the rat brain for reference (Franklin and Paxinos 1998). All sensors were verified to be positioned within the prelimbic or infralimbic part of the prefrontal cortex at 3.2±0.4 mm anterior of bregma, the shell or core part of the nucleus accumbens at 1.85±0.4 mm anterior of bregma or the CA1 or CA2 part of the hippocampus at 5.2±0.4 mm posterior of bregma.

Data and statistical analysis

The NO oxidation current over time (sampling rate 4/s) recorded in chart was used as data. The mean of the sampling period (approximately 5 min) immediately prior to drug treatment was used as a baseline. The mean current change from the baseline was calculated for each 4-min sampling period corresponding to a PPI test.

PPI was analysed by calculating the mean response amplitude for pulse-alone trials (P) for each rat 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:

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

Using this formula, a 0% value denotes no difference between pulse-alone and prepulse pulse response amplitudes and consequently no PPI.

Differences in NO levels between treatments (saline or LNPA+saline and PCP or LNPA+PCP) were assessed using a two-way repeated measures ANOVA with sampling time as within-subjects factor and treatment as between-subjects factor. For the PPI data, a two-way repeated measures ANOVA with sampling time as within-subjects factor and treatment as between-subjects factor including all animals in experiment 1 was performed. This was followed by a two-way ANOVA of the mean of the first and last four PPI tests with treatment and brain region as between-subjects factors. Experiment 2 was analysed using a two-way ANOVA of the mean of the first and last four PPI tests with treatment and dose as between-subjects factors. Group differences were further evaluated using Bonferroni's post hoc test. Two-tailed levels of significance were used and $p<0.05$ was considered statistically significant.

Results

Prefrontal cortex NO levels PCP (2 mg/kg) administration

The ANOVA showed that systemic administration of PCP induced an increase in NO levels (effect of treatment, $F(1,154)=6.91$, $p<0.05$) that was time-dependent (treatment× time interaction, $F(11,154)=6.69$, $p<0.001$). The post hoc test indicated a significant effect of PCP on NO levels during the 108-min ($p<0.05$), 122-min ($p<0.01$), 136-min ($p<0.01$), 154-min ($p<0.001$) and 164-min ($p<0.01$) sampling times (Fig. 1a).

Ventral hippocampus NO levels and PPI following PCP (2 mg/kg) administration

The two-way ANOVA demonstrated a significant time-dependent increase in NO levels following PCP-induced administration (treatment×time interaction, $F(11,110)=2.04$, $p<0.05$; Fig. 1b).

Nucleus accumbens NO levels and PPI following PCP (2 mg/kg) administration

The two-way ANOVA did not reveal any main effects for the time and treatment factors and no significant interaction effect (Fig. 1c).

Prefrontal cortex, ventral hippocampus and nucleus accumbens PPI following PCP (2 mg/kg) administration

The analysis of the PPI for all animals in experiment 1 showed a significant decrease in PPI after PCP treatment (effect of treatment, $F(1,418)=21.68$, $p<0.0001$) that was time-dependent (treatment×time interaction, $F(11,418)=$

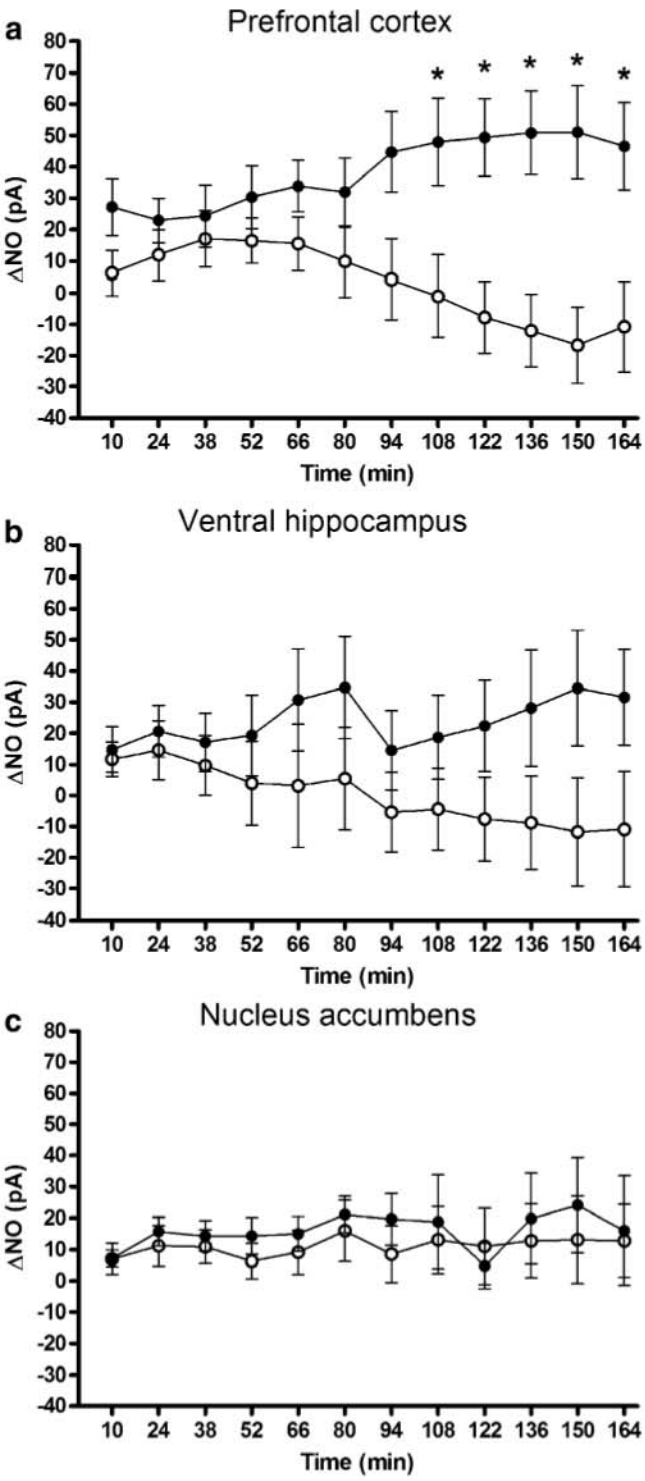

Fig. 1 Mean change in prefrontal cortex ($n=8$) (**a**), ventral hippocampus ($n=6$) (**b**) and nucleus accumbens ($n=6$) (**c**) NO oxidation current as compared to baseline for each 4-min sampling period corresponding to a PPI test. All animals received the two treatments in a semi-randomised order with a 2-day washout period between the saline (*open circles*) and PCP (*filled circles*) condition. Data are expressed as mean ± SEM current change (pA). *$p<0.05$ following a Bonferroni post hoc test

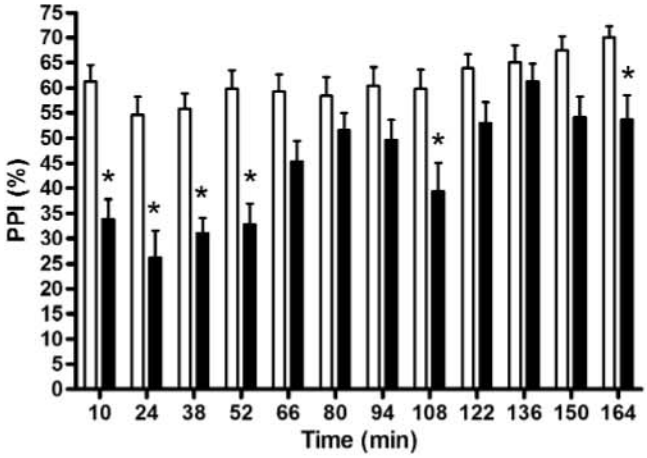

Fig. 2 Change in PPI levels for each 4-min sampling period. All animals in experiment 1 (n=20) received the two treatments in a semi-randomised order with a 2-day washout period between the saline (*open bars*) and PCP (*filled bars*) condition. Data are expressed as mean ± SEM PPI (%). *p<0.05 following a Bonferroni post hoc test

4.12, p<0.0001). Furthermore, there was a main effect of time per se on PPI levels (effect of time, F(11,418)=12.86, p<0.0001). The Bonferroni post hoc test demonstrated a significant group difference during the 10-min (p<0.001), 24-min (p<0.001), 38-min (p<0.001), 52-min (p<0.001), 108-min (p<0.01) and 164-min (p<0.05) sampling times (Fig. 2).

Furthermore, a two-way ANOVA of the mean PPI during the first four PPI tests showed a main effect of treatment (F(1,34)=36.52, p<0.0001), but no effect of brain region or any treatment×brain region interaction effect (Fig. 3a). The Bonferroni post hoc test demonstrated a significant difference between saline and PCP treatment for all brain regions, prefrontal cortex (p<0.001), ventral hippocampus (p<0.05) and nucleus accumbens (p<0.01).

A similar analysis of the mean PPI during the last four PPI tests also showed a main effect of treatment (F(1,34)= 8.21, p<0.01), but no effect of brain region or any treatment×brain region interaction effect (Fig. 3b). Furthermore, the Bonferroni post hoc test did not reveal any significant differences in PPI between saline and PCP treatment groups.

Prefrontal cortex NO levels following LNPA (5 mg/kg) and PCP (2 mg/kg) administration

The analysis indicated that systemic administration of PCP increased NO levels (effect of treatment, F(1,110)=6.14, p<0.05) in a time-dependent manner (treatment×time interaction, F(11,110)=3.12, p<0.01). The post hoc test indicated a significant effect of PCP on NO levels during the 122-min (p<0.05), 136-min (p<0.01) and 154-min (p< 0.05) sampling times (Fig. 4a).

Prefrontal cortex NO levels following LNPA (10 mg/kg) and PCP (2 mg/kg) administration

The two-way ANOVA did not show any main effect of treatment or time×treatment interaction, indicating that LNPA pretreatment attenuated a PCP-induced increase in NO levels (Fig. 4b). However, there was a main effect of time (effect of time, F(11,110)=3.43, p<0.01), suggesting that pretreatment with LNPA (10 mg/kg) decreased NO levels per se.

PPI levels following LNPA (5 or 10 mg/kg) and PCP (2 mg/kg) administration

The two-way ANOVA of the mean PPI during the first four PPI tests did not show any main effect of treatment, dose or any treatment×dose interaction effect (Fig. 5a). Similarly, the analysis of the mean PPI during the last four PPI tests did not demonstrate any treatment, dose or any treatment× dose interaction effect (Fig. 5b).

Acoustic startle response

A t test was used to compare the mean acoustic startle response between treatment groups within each experiment,

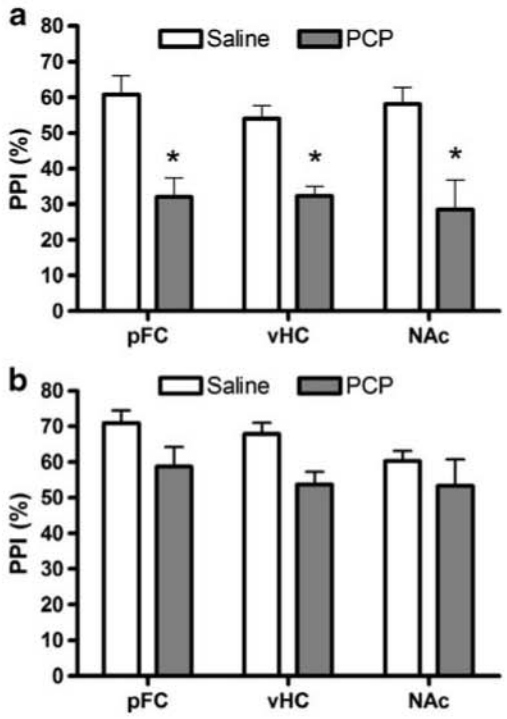

Fig. 3 Mean change in PPI levels for the first (10–52 min) (**a**) and last (122–164 min) (**b**) sampling periods. All animals (n=6–8) received the two treatments in a semi-randomised order with a 2-day washout period between the saline (*open bars*) and PCP (*filled bars*) condition. Data are expressed as mean ± SEM PPI (%). *p<0.05 following a Bonferroni post hoc test

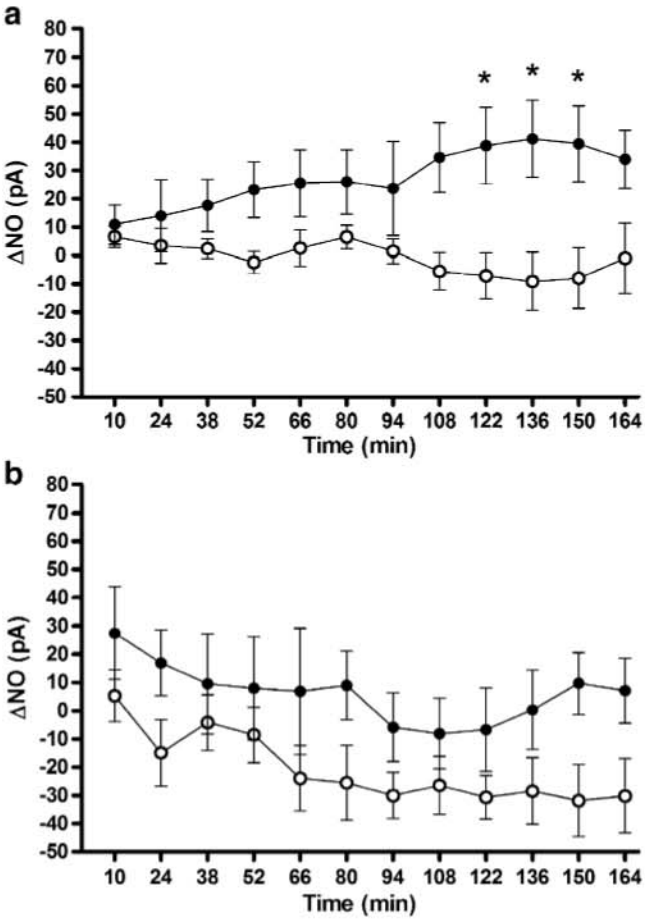

Fig. 4 **a** Mean change in prefrontal cortex NO oxidation current as compared to baseline for each 4-min sampling period corresponding to a PPI test. All animals (n=6) received the two treatment combinations in a semi-randomised order with a 2-day washout period between LNPA (5 mg/kg)+saline (*open circles*) and LNPA (5 mg/kg)+PCP (*filled circles*) condition. Data are expressed as mean ± SEM current change (pA). *p<0.05 following a Bonferroni post hoc test. **b** Mean change in NO oxidation current as compared to baseline for each 4-min sampling period corresponding to a PPI test. All animals (n=6) received the two treatment combinations in a semi-randomised order with a 2-day washout period between the LNPA (10 mg/kg)+saline (*open circles*) and LNPA (10 mg/kg)+PCP (*filled circles*) condition. Data are expressed as mean ± SEM current change (pA). *p<0.05 following a Bonferroni post hoc test

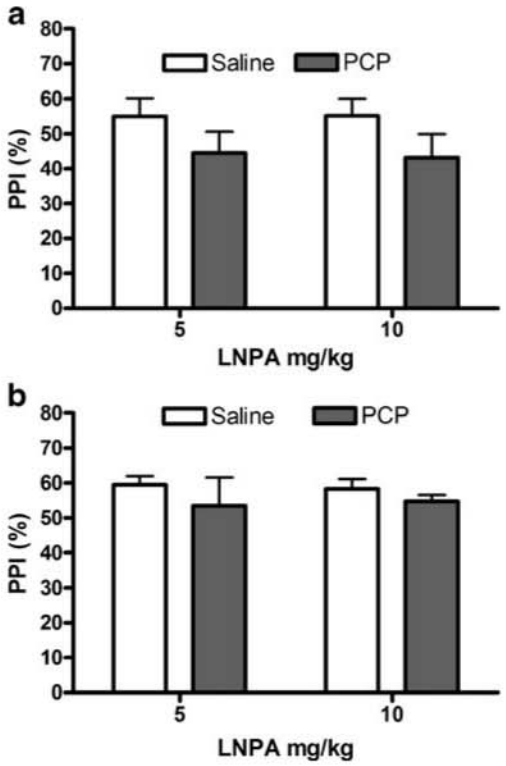

Fig. 5 Mean change in PPI levels for the first (10–52 min) (**a**) and last (122–164 min) (**b**) sampling periods. All animals (n=6) received the two treatments in a semi-randomised order with a 2-day washout period between the LNPA+Saline (*open bars*) and LNPA+PCP (*filled bars*) condition. Data are expressed as mean ± SEM PPI (%). *p<0.05 following a Bonferroni post hoc test

and no significant effect of treatment was found in any experiment (Table 1).

Discussion

The present study confirms a previously observed increase in NO levels in the prefrontal cortex of rats following systemic PCP administration (Palsson et al. 2009). In addition, this effect tended to be more pronounced in the prefrontal cortex as compared to the ventral hippocampus, whereas no elevation of NO levels was found in the nucleus accumbens. The fact that PCP did not elicit an NO release in the nucleus accumbens may be due to regional differences in the anatomy and regulation of the NO system in the brain (Steinbusch et al. 2000). Furthermore, systemic administration of PCP affects a number of neurotransmitter systems in several brain regions. This includes elevations of dopamine and serotonin levels and turnover (Jentsch et al. 1997; Martin et al. 1998). These effects have been linked to the PPI

Table 1 Mean acoustic startle response for each experiment and treatment condition (n=6–8)

Experiment	Saline	PCP (2mg/kg)	t test
Prefrontal cortex	1,181±92	1,216±95	ns
Ventral hippocampus	1,233±99	1,228±203	ns
Nucleus accumbens	1,230±148	1,057±172	ns
LNPA 5 mg/kg	989±162	1,009±55	ns
LNPA 10 mg/kg	948±133	912±102	ns

Data are expressed as mean ± SEM startle response (digital units)

deficit induced by PCP (Geyer et al. 2001), and both serotonin and dopamine interact with NO signalling (Wegener et al. 2000). However, systemic administration of PCP or its analogue MK-801 increases both the prefrontal cortex and nucleus accumbens dopamine and serotonin levels (Hernandez et al. 1988; Loscher et al. 1991). Thus, changes in serotonin and dopamine levels are unlikely to explain the observed difference between the prefrontal cortex and the nucleus accumbens. It has been demonstrated that the NO system is regulated by NMDA receptor activity in the striatum (Park and West 2009), whereas prefrontal cortex NO activity is more dependent on GABA receptors (Pepicelli et al. 2004). In support of this, it was recently shown that a PCP-induced deficit in PPI could be ameliorated by the $GABA_B$ agonist baclofen in a dose that also lowered prefrontal cortex NO levels (Fejgin et al. 2009). For the hippocampus, both GABAergic and glutamatergic modulation of NO signalling has been described (Fedele et al. 2001). It is unclear from the literature if systemic administration of PCP affects hippocampal dopamine levels; if anything, PCP has been shown to antagonise NMDA receptor-dependent release of dopamine in a hippocampal slice preparation (Chaki et al. 1998). Furthermore, it has been suggested that the main interaction between PCP and serotonin occurs in the dorsal and not ventral part of the hippocampus (Kusljic and van den Buuse 2004). In summary, the broad pharmacological profile of PCP and its effect on a number of brain regions makes it difficult to draw any definite conclusions regarding the mechanism behind the observed effects on NO levels. The observed difference between the three investigated brain regions could suggest that a GABAergic and not NMDA, dopamine or serotonin receptor mechanism is primarily involved. However, PCP interacts with other neurotransmitter systems, and interactions between different receptor populations should also be considered.

An additional observation from the present study is that the increase in prefrontal cortex NO was more prolonged as compared to similar experiments where animals were administered PCP in their home cage (Palsson et al. 2009). Whether this observation is a result of the different experimental procedures, i.e. home cage versus PPI tests, or simply a result of variability between animals remains to be determined. However, recent studies show an important effect of oxidative stress in the neurotoxic effects of PCP analogues (Behrens and Sejnowski 2009), and NO has the potential to both increase and protect against oxidative stress toxicity (Guix et al. 2005). Furthermore, previous work has shown that both acute and subchronic administration of PCP can potentiate the behavioural response to stressful stimuli and increase stress-dependent brain c-fos immunoreactivity (Turgeon et al. 2007). In addition, NO may activate the HPA axis (Lopez-Figueroa et al. 1998), and NOS inhibitors attenuate stress-induced behaviour in rats (Sevgi et al. 2006). Thus, the present observation may reflect an interaction between PCP and a stress response converging on the NO signalling pathway.

The present data also support the involvement of neuronal NOS in the schizophrenia-related behavioural effects of PCP. The neuronal NOS inhibitor LNPA attenuated a PCP-induced deficit in PPI and dose-dependently reduced a PCP-induced increase in prefrontal cortex NO levels. The time course (Klamer et al. 2005a) and neuronal NOS dependence (Klamer et al. 2004a) of the PCP-induced deficit in PPI are in agreement with previous findings. However, the lack of correlation between NO levels and the behavioural effects of PCP stand in contrast to earlier published work (Palsson et al. 2009). A number of NOS inhibitors (Johansson et al. 1997; Wiley 1998), as well as inhibition of cGMP signalling (Fejgin et al. 2008), have been shown to attenuate a PCP-induced deficit in PPI. Furthermore, neuronal NOS knockdown mice do not show a deficit in PPI when challenged with PCP (Klamer et al. 2004c), and thus, it may be hypothesised that NO/sGC/cGMP signalling plays an important role in mediating some of the behavioural effects of PCP. However, the present findings demonstrate peak effects of PCP on NO levels that are temporally displaced with regards to the behavioural effects. Furthermore, a low dose of LNPA (5 mg/kg) normalised PPI without fully blocking the induction of the NO system. Thus, it would appear that increased NO levels are not directly associated with the PPI disruption induced by PCP. Interestingly, a similar temporal disassociation has been observed for increased prefrontal cortex dopamine and glutamate levels and cognitive dysfunction after PCP administration (Adams and Moghaddam 1998). Increased glutamate levels may reflect a cortical disinhibition caused by PCP, involving GABAergic interneurons (Homayoun and Moghaddam 2007). Importantly, within the prefrontal cortex, NOS is primarily expressed in a subpopulation of GABAergic neurons that target both pyramidal cells and other interneurons (Vruwink et al. 2001). Thus, NO is well positioned to influence cortical activity both though synaptic and volume transmission actions. However, the lack of correlation between PCP-induced increases in prefrontal cortex NO levels and deficit in PPI remains to be reconciled with the beneficial effect of NOS inhibitors on the said deficit.

The in vivo NO levels as measured by amperometry may not accurately mirror actual synaptic NO signalling. The design of the NO sensors used in the current study, and amperometric electrodes generally employed for neurochemical analysis (~5 300 μm in diameter), means that they monitor an average concentration of analyte in the ECF and not directly in synapses that are orders of

magnitude smaller (typically <50 nM; Lowry and O'Neill 2005). Thus, the observed effects likely reflect volume transmission of NO rather than synaptic transmission. Conceivably, synaptic NO levels are also increased but remain below the limit of detection using our method. To accurately monitor NO signalling at the synaptic level, other methods are likely needed (Sato et al. 2005). Furthermore, the actual in vivo levels of NO have never been accurately determined, although many studies suggest that low nanomolar levels would be the likely physiological range (Hall and Garthwaite 2009). Furthermore, enzyme-based inactivation of NO has been demonstrated in vitro, which may limit the intercellular diffusion of NO (Hall et al. 2009). Coupled with the known clearance of NO by haemoglobin, there appears to be strong constraints on the movement and half-life of NO in the cellular environment. The observable increase in NO levels following PCP administration may reflect targeted extracellular release of NO or spillover from synaptic NO signalling, although the latter seems unlikely given the time course and the magnitude of the increase (Hall and Garthwaite 2009). Volume transmission has been demonstrated for NO in vitro (Steinert et al. 2008), but further studies are clearly needed to accommodate this phenomenon with models of synaptic NO signalling (Garthwaite 2010) and NO clearance in vivo (Hall and Garthwaite 2006).

In conclusion, the present set of experiments support a previously demonstrated role of NO in the behavioural and neurochemical effects of PCP. Furthermore, this effect is brain region-specific and appears to mainly involve the neuronal isoform of NOS, but the present study also fails to demonstrate a temporal correlation between a PCP-induced disruption of PPI and an increase in prefrontal cortex NO levels. In addition, evidence of an interaction between PCP and stressful stimuli on prefrontal cortex NO levels was found. A PPI test in combination with PCP administration increased NO levels more potently than what was observed in a previous study where PCP was administered during rest. Taken together, these data suggest that the interaction between PCP and the NO system is more complex than suggested by previous work. Further studies are needed to fully elucidate the mechanism of action and functional role of NO in the behavioural and neurochemical effects of PCP.

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References

Adams B, Moghaddam B (1998) Corticolimbic dopamine neurotransmission is temporally dissociated from the cognitive and locomotor effects of phencyclidine. J Neurosci 18:5545–5554

Baba H, Suzuki T, Arai H, Emson PC (2004) Expression of nNOS and soluble guanylate cyclase in schizophrenic brain. NeuroReport 15:677–680

Behrens MM, Sejnowski TJ (2009) Does schizophrenia arise from oxidative dysregulation of parvalbumin-interneurons in the developing cortex? Neuropharmacology 57:193–200

Bernstein HG, Krell D, Braunewell KH, Baumann B, Gundelfinger ED, Diekmann S, Danos P, Bogerts B (2001) Increased number of nitric oxide synthase immunoreactive Purkinje cells and dentate nucleus neurons in schizophrenia. J Neurocytol 30:661–670

Bird DC, Bujas-Bobanovic M, Robertson HA, Dursun SM (2001) Lack of phencyclidine-induced effects in mice with reduced neuronal nitric oxide synthase. Psychopharmacology (Berl) 155:299–309

Braff DL, Geyer MA, Light GA, Sprock J, Perry W, Cadenhead KS, Swerdlow NR (2001) Impact of prepulse characteristics on the detection of sensorimotor gating deficits in schizophrenia. Schizophr Res 49:171–178

Brown FO, Lowry JP (2003) Microelectrochemical sensors for in vivo brain analysis: an investigation of procedures for modifying Pt electrodes using Nafion. Analyst 128:700–705

Brown FO, Finnerty NJ, Bolger FB, Millar J, Lowry JP (2005) Calibration of NO sensors for in-vivo voltammetry: laboratory synthesis of NO and the use of UV–visible spectroscopy for determining stock concentrations. Anal Bioanal Chem 381:964–971

Brown FO, Finnerty NJ, Lowry JP (2009) Nitric oxide monitoring in brain extracellular fluid: characterisation of Nafion-modified Pt electrodes in vitro and in vivo. Analyst 134:2012–2020

Bujas-Bobanovic M, Bird DC, Robertson HA, Dursun SM (2000) Blockade of phencyclidine-induced effects by a nitric oxide donor. Br J Pharmacol 130:1005–1012

Chaki S, Okuyama S, Ogawa S, Tomisawa K (1998) Regulation of NMDA-induced [^{3}H]dopamine release from rat hippocampal slices through sigma-1 binding sites. Neurochem Int 33:29–34

Deutsch SI, Rosse RB, Schwartz BL, Fay-McCarthy M, Rosenberg PB, Fearing K (1997) Methylene blue adjuvant therapy of schizophrenia. Clin Neuropharmacol 20:357–363

Fedele E, Marchi M, Raiteri M (2001) In vivo NO/cGMP signalling in the hippocampus. Neurochem Res 26:1069–1078

Fejgin K, Palsson E, Wass C, Svensson L, Klamer D (2008) Nitric oxide signaling in the medial prefrontal cortex is involved in the biochemical and behavioral effects of phencyclidine. Neuropsychopharmacology 33:1874–1883

Fejgin K, Palsson E, Wass C, Finnerty N, Lowry J, Klamer D (2009) Prefrontal GABA(B) receptor activation attenuates phencyclidine-induced impairments of prepulse inhibition: involvement of nitric oxide. Neuropsychopharmacology 34:1673–1684

Franklin KBJ, Paxinos G (1998) The rat brain in stereotaxic coordinates. Academic Press: New York

Garthwaite J (2010) New insight into the functioning of nitric oxide-receptive guanylyl cyclase: physiological and pharmacological implications. Mol Cell Biochem 334:221–232

Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR (2001) Pharmacological studies of prepulse inhibition models of

sensorimotor gating deficits in schizophrenia: a decade in review. Psychopharmacology (Berl) 156:117–154
Goff DC, Cather C, Freudenreich O, Henderson DC, Evins AE, Culhane MA, Walsh JP (2009) A placebo-controlled study of sildenafil effects on cognition in schizophrenia. Psychopharmacology (Berl) 202:411–417
Guix FX, Uribesalgo I, Coma M, Munoz FJ (2005) The physiology and pathophysiology of nitric oxide in the brain. Prog Neurobiol 76:126–152
Hall CN, Garthwaite J (2006) Inactivation of nitric oxide by rat cerebellar slices. J Physiol 577:549–567
Hall CN, Garthwaite J (2009) What is the real physiological NO concentration in vivo? Nitric Oxide 21:92–103
Hall CN, Keynes RG, Garthwaite J (2009) Cytochrome P450 oxidoreductase participates in nitric oxide consumption by rat brain. Biochem J 419:411–418
Hernandez L, Auerbach S, Hoebel BG (1988) Phencyclidine (PCP) injected in the nucleus accumbens increases extracellular dopamine and serotonin as measured by microdialysis. Life Sci 42:1713–1723
Homayoun H, Moghaddam B (2007) NMDA receptor hypofunction produces opposite effects on prefrontal cortex interneurons and pyramidal neurons. J Neurosci 27:11496–11500
Jentsch JD, Elsworth JD, Redmond DE Jr, Roth RH (1997) Phencyclidine increases forebrain monoamine metabolism in rats and monkeys: modulation by the isomers of HA966. J Neurosci 17:1769–1775
Johansson C, Jackson DM, Svensson L (1997) Nitric oxide synthase inhibition blocks phencyclidine-induced behavioural effects on prepulse inhibition and locomotor activity in the rat. Psychopharmacology (Berl) 131:167–173
Klamer D, Engel JA, Svensson L (2001) The nitric oxide synthase inhibitor, L-NAME, block phencyclidine-induced disruption of prepulse inhibition in mice. Psychopharmacology (Berl) 156:182–186
Klamer D, Engel JA, Svensson L (2004a) The neuronal selective nitric oxide synthase inhibitor, Nomega-propyl-L-arginine, blocks the effects of phencyclidine on prepulse inhibition and locomotor activity in mice. Eur J Pharmacol 503:103–107
Klamer D, Engel JA, Svensson L (2004b) Phencyclidine-induced behaviour in mice prevented by methylene blue. Pharmacol Toxicol 94:65–72
Klamer D, Engel JA, Svensson L (2004c) Phencyclidine increases prepulse inhibition of acoustic startle in neuronal nitric oxide synthase deficient mice. The Journal of the European College of Neuropsychopharmacology 14(Supplement 1):36–37
Klamer D, Palsson E, Revesz A, Engel JA, Svensson L (2004d) Habituation of acoustic startle is disrupted by psychotomimetic drugs: differential dependence on dopaminergic and nitric oxide modulatory mechanisms. Psychopharmacology (Berl) 176:440–450
Klamer D, Palsson E, Fejgin K, Zhang J, Engel JA, Svensson L (2005a) Activation of a nitric-oxide-sensitive cAMP pathway with phencyclidine: elevated hippocampal cAMP levels are temporally associated with deficits in prepulse inhibition. Psychopharmacology (Berl) 179:479–488
Klamer D, Palsson E, Wass C, Archer T, Engel JA, Svensson L (2005b) Antagonism of the nitric oxide synthase inhibitor, L-NAME, of the effects of phencyclidine on latent inhibition in taste aversion conditioning. Behav Brain Res 161:60–68
Kusljic S, van den Buuse M (2004) Functional dissociation between serotonergic pathways in dorsal and ventral hippocampus in psychotomimetic drug-induced locomotor hyperactivity and prepulse inhibition in rats. Eur J Neurosci 20:3424–3432
Lopez-Figueroa MO, Day HE, Akil H, Watson SJ (1998) Nitric oxide in the stress axis. Histol Histopathol 13:1243–1252
Loscher W, Annies R, Honack D (1991) The *N*-methyl-D-aspartate receptor antagonist MK-801 induces increases in dopamine and serotonin metabolism in several brain regions of rats. Neurosci Lett 128:191–194
Lowry JP, O'Neill RD (2005) Neuroanalytical chemistry in vivo using biosensors. In: Grimes CA, Dickey EC (eds) Encyclopedia of sensors. American Scientific Publishers, California, pp 501–524
Lowry JP, Boutelle MG, Fillenz M (1997) Measurement of brain tissue oxygen at a carbon past electrode can serve as an index of increases in regional cerebral blood flow. J Neurosci Methods 71:177–182
Martin P, Carlsson ML, Hjorth S (1998) Systemic PCP treatment elevates brain extracellular 5-HT: a microdialysis study in awake rats. NeuroReport 9:2985–2988
Palsson E, Finnerty N, Fejgin K, Klamer D, Wass C, Svensson L, Lowry J (2009) Increased cortical nitric oxide release after phencyclidine administration. Synapse 63:1083–1088
Park DJ, West AR (2009) Regulation of striatal nitric oxide synthesis by local dopamine and glutamate interactions. J Neurochem 111:1457–1465
Pepicelli O, Brescia A, Gherzi E, Raiteri M, Fedele E (2004) GABA (A), but not NMDA, receptors modulate in vivo NO-mediated cGMP synthesis in the rat cerebral cortex. Neuropharmacology 46:480–489
Reif A, Herterich S, Strobel A, Ehlis AC, Saur D, Jacob CP, Wienker T, Topner T, Fritzen S, Walter U, Schmitt A, Fallgatter AJ, Lesch KP (2006) A neuronal nitric oxide synthase (NOS-I) haplotype associated with schizophrenia modifies prefrontal cortex function. Mol Psychiatry 11:286–300
Sato M, Hida N, Umezawa Y (2005) Imaging the nanomolar range of nitric oxide with an amplifier-coupled fluorescent indicator in living cells. Proc Natl Acad Sci USA 102:14515–14520
Sevgi S, Ozek M, Eroglu L (2006) L-NAME prevents anxiety-like and depression-like behavior in rats exposed to restraint stress. Methods Find Exp Clin Pharmacol 28:95–99
Steinbusch HWM, De Vente J, Vincent SR (2000) Functional neuroanatomy of the nitric oxide system. In: Björklund A, Hökfelt T (eds) Handbook of chemical neuroanatomy. Elsevier, Amsterdam
Steinert JR, Kopp-Scheinpflug C, Baker C, Challiss RA, Mistry R, Haustein MD, Griffin SJ, Tong H, Graham BP, Forsythe ID (2008) Nitric oxide is a volume transmitter regulating postsynaptic excitability at a glutamatergic synapse. Neuron 60:642–656
Swerdlow NR, Weber M, Qu Y, Light GA, Braff DL (2008) Realistic expectations of prepulse inhibition in translational models for schizophrenia research. Psychopharmacology (Berl) 199:331–388
Turgeon SM, Lin T, Subramanian M (2007) Subchronic phencyclidine exposure potentiates the behavioral and c-Fos response to stressful stimuli in rats. Pharmacol Biochem Behav 88:73–81
Wass C, Archer T, Palsson E, Fejgin K, Alexandersson A, Klamer D, Engel JA, Svensson L (2006) Phencyclidine affects memory in a nitric oxide-dependent manner: working and reference memory. Behav Brain Res 174:49–55
Wass C, Klamer D, Fejgin K, Palsson E (2009) The importance of nitric oxide in social dysfunction. Behav Brain Res 200:113–116
Wegener G, Volke V, Rosenberg R (2000) Endogenous nitric oxide decreases hippocampal levels of serotonin and dopamine in vivo. Br J Pharmacol 130:575–580
Wiley JL (1998) Nitric oxide synthase inhibitors attenuate phencyclidine-induced disruption of prepulse inhibition. Neuropsychopharmacology 19:86–94
Wratten NS, Memoli H, Huang Y, Dulencin AM, Matteson PG, Comacchia MA, Azaro MA, Messenger J, Hayter JE, Bassett AS, Buyske S, Millonig JH, Vieland VJ, Brzustowicz LM (2009) Identification of a schizophrenia-associated functional noncoding variant in NOS1AP. Am J Psychiatry 166:434–441
Vruwink M, Schmidt HH, Weinberg RJ, Burette A (2001) Substance P and nitric oxide signaling in cerebral cortex: anatomical evidence for reciprocal signaling between two classes of interneurons. J Comp Neurol 441:288–301