2-1-2014

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International Journal of Neuropsychopharmacology (2014), **17**, 303–312. © CINP 2013
doi:10.1017/S1461145713001041

ARTICLE

Activation of mGluR2/3 receptors in the ventro-rostral prefrontal cortex reverses sensorimotor gating deficits induced by systemic NMDA receptor antagonists

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Abstract

Prepulse inhibition (PPI) of acoustic startle is an operational measure of sensorimotor gating, which is disrupted in schizophrenia. NMDA receptor (NMDAR) antagonist induced PPI disruption has become an important pharmacological model for schizophrenia; however, knowledge of the underlying mechanism remains incomplete. This study examines the role of NMDAR in the caudal pontine reticular nucleus (PnC) and the medial prefrontal cortex (mPFC) in NMDARs antagonist induced PPI deficits, as well as the NMDA receptor subtypes involved. We administered the NMDA antagonist MK-801 locally into the caudal pontine reticular formation (PnC), where the PPI mediating pathway converges with the primary startle pathway, and into the mPFC prior to behavioural testing. PnC microinjections had no effect on startle and PPI, whereas injections into the ventro-rostral part, but not into the dorso-caudal part of the mPFC, disrupted PPI. These effects could be mimicked by local injection of the NR2B subunit specific antagonist ifenprodil, whereas co-application of MK-801 and the mGluR2/3 agonist LY354740 had no effect on PPI. Moreover, PPI disruptions by systemically administered MK-801 could be reversed by local injections of LY354740 into the ventro-rostral mPFC, but not into the dorso-caudal mPFC. Our results indicate that NR2B subunit containing NMDARs in a specific subregion of the mPFC play a major role in PPI disruptions by systemic NMDAR antagonism. Our results further support the hypothesis that glutamate hyper-function in the mPFC is a main mechanism involved in sensory gating deficits induced by systemic MK-801, supporting the notion that this is an important mechanism in schizophrenia pathology.

Received 15 April 2013; Reviewed 21 May 2013; Revised 2 August 2013; Accepted 5 August 2013; First published online 26 September 2013

Key words: Glutamate receptor, Prepulse inhibition, Rats, Startle, Schizophrenia.

Introduction

Sensorimotor gating is a pre-attentive mechanism that can be measured as prepulse inhibition (PPI) of acoustic startle responses in humans and in animal models (Geyer and Braff, 1987; Braff and Geyer, 1990; Braff et al., 1992). It describes the inhibition of a startle response to a sudden loud noise stimulus by a preceding prepulse. This prepulse can be any sensory stimulus that does not elicit a startle reaction itself; often a low volume acoustic stimulus is used. Sensorimotor gating deficits, and hence disruption of PPI, are hallmarks of schizophrenia and can also be observed in other neurological disorders (Geyer and Braff, 1987; Swerdlow et al., 1995, 2000). In animal models, the administration of dopamine agonists induces PPI deficits (Mansbach et al., 1988), which are reversed by antipsychotic drugs. In people with schizophrenia, however, antipsychotics often do not, or only partly, reverse PPI deficits. Since PPI deficits are strongly associated with other cognitive disruptions (Swerdlow et al., 2006; Singer et al., 2013), drug targets other than the dopaminergic system are sought in order to address this group of schizophrenia symptoms. The most promising alternative model is the glutamatergic schizophrenia model. This model is based on the observation that the administration of non-competitive NMDAR antagonists such as phencyclidine (PCP) induces not only the positive, but also the negative and cognitive symptoms of schizophrenia in humans (Javitt, 1987; Javitt and Zukin, 1991; Krystal et al., 1994) and PPI deficits in rats (Mansbach and Geyer, 1991; Moghaddam and Bolinao, 1994). It is thought that NMDAR antagonism may simulate NMDAR hypofunction in the mPFC associated with schizophrenia, thus producing similar effects on behavioural and cognitive processes (Moghaddam and Javitt, 2012; see also O'Donnell, 2012). Further investigations have revealed evidence that NMDAR hypo-function results in an increased

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glutamate release in the medial prefrontal cortex (mPFC) and excitation of non-NMDARs, and that this effect can be reversed by inhibition of glutamate release, e.g. by the activation of mGluR2/3 receptors (Olney and Farber, 1995; Moghaddam et al., 1997; Jackson et al., 2004; Homayoun and Moghaddam, 2007). The particular importance of the mPFC in the glutamatergic schizophrenia model has also been implicated through a study by Schwabe and Koch (2004), showing that NMDAR antagonism fails to induce PPI deficits in mPFC lesioned animals. NMDARs, however, are abundantly expressed in the brain, including in areas directly implicated in mediating startle and PPI. Startle mediating neurons in the pontine reticular formation receive glutamatergic sensory and modulatory input that activates different glutamate receptors, including NMDARs (Weber et al., 2002; Schmid et al., 2010). Here, we addressed the hypothesis that systemic NMDAR antagonists exert their main effects on sensorimotor gating through NMDAR inhibition in the mPFC, rather than on neuronal pathways directly involved in prepulse inhibition of startle. We further tested whether NR2B subunit containing NMDARs are involved, and whether the inhibition of glutamate release in the mPFC through the local administration of the mGluR2/3 agonist LY354740 can reverse PPI deficits induced by systemically administered MK-801.

Method

Animals

Sprague–Dawley rats obtained from Charles River® (Canada) were used for all experiments. Rats weighed about 250–300 g at the time of surgery, and about 300–350 g at the time of testing. Animals were group housed until surgery in clear plastic caging with *ad libitum* access to rat chow and water, and individually housed following surgery to prevent injury to the animals and damage to the implants. Animals were kept on a 12:12 h light–dark cycle with transitions at 07.00 hours and 19.00 hours, in a temperature controlled room kept at 23 °C. All animal procedures were approved by the University of Western Ontario Animal Use Committee, and complied with the ethical guidelines of the Canadian Council on Animal Care involving vertebrate animals in research. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Stereotaxic surgery

Two to five days following arrival, rats underwent surgery to implant bilateral chronic indwelling cannulae (28 gauge, PlasticsOne, USA) targeted bilaterally to either the PnC or mPFC. Animals were anesthetized via inhalation of 2% vapourized isoflurane (Forane) and 98% oxygen delivered to the nose cone attached to the stereotaxic apparatus (Stoelting, USA). They also received injections of 0.05 mg/kg buprenorphine and 2.5 mg/kg ketoprofen for analgesia, and an injection of 5 ml/kg 0.9% sterile saline to ensure proper hydration during recovery. Cannula coordinates were as follows: mPFC (DV: −3.2 to −3.7 mm from skull surface; ML: ±0.6 mm, from midline; RC: +2.7 to +3.2 mm from bregma) and PnC (DV: −8.50 mm, from skull surface; ML: ±2.50 mm, from midline; RC: −2.00 mm, from lambda; cannulae were laterally angled at 10° in order to avoid severing the choroid plexus). The cannulae were anchored to the skull using four stainless steel bone screws and dental cement, and the wound was closed using silk suture. Stainless steel stylets (PlasticsOne) were inserted to keep the cannulae free of obstructions. After surgery, animals were allowed to heal for 4–7 d before handling.

Initial handling and exposure to behavioural paradigm

Handling was done daily for 1–2 min per rat to replace stylets, and habituate the subjects to handling. The rats were handled and tickled to increase their affinity for their handlers (Burgdorf & Panksepp, 2001). Rats were handled for three consecutive days before any injection to ensure habituation to being handled by the experimenter. All subjects were also placed in the startle boxes on the day before testing for one exposure to the behavioural paradigm and confinement in the startle boxes.

Drugs

The general non-competitive NMDAR antagonist MK-801 (Sigma, USA), and the NR2B subtype specific, non-competitive NMDA antagonist ifenprodil (Sigma) were dissolved in 0.9% saline. The mGluR2/3 agonist LY 354740 (Tocris, UK) was dissolved in 0.9% saline with 1 eq. NaOH. All drugs were maintained at −18 °C following dissolution. MK-801 was administered systemically (0.01, 0.1, 1.0 mg/kg, i.p.) and intracranially (i.c.) (5, 50 mM). Ifenprodil was administered i.c. (100, 1 mM). LY 354740 was administered i.c. (500 μM). All doses used are within the range of well-established doses in the literature (systemic MK-801: Geyer et al., 2001; Bortolato et al., 2004; Schwabe and Koch, 2004; MK-801 microinfusion: Bakshi and Geyer, 1998; Bakshi & Geyer 1999; Zhang et al., 2000; Figueroa-Guzmán et al., 2006; ifenprodil microinfusion: Ma et al., 2007; Day et al., 2011; Blair et al., 2005; LY 354740 microinfusion: Jackson & Moghaddam 2001; Mela et al., 2006). In general, doses for microinfusions were 100–1000 times the minimal effective dose in order to account for dilution by the extracellular fluid.

Intracranial drug infusion

Each animal got one saline injection and either one or two drug injections, with at least 5 d in between injections. Order of saline and drug injections was pseudorandomized. Injections of 0.5 μl of saline or drug per site were done using 5 μl Hamilton micro-syringes mounted on a

motorized pump (World Precision Instruments, USA). Injections occurred over a period of 4 min, and the injectors were left in place for an additional 1–2 min in order to ensure that the drug dispersed and did not flow back within the cannulae. Following injections, stylets were sterilized and replaced, and animals were returned to their home cages.

Behavioural testing

Behavioural testing was done in startle boxes (Med Associates, USA) using the Startle reflex 5.95 software (Med Associates). The behavioural testing paradigm consisted of the following phases: the acclimation phase, a habituation phase (block 1), and PPI measurement (block 2, see also Valsamis and Schmid, 2011). During the acclimation phase animals were exposed to the chambers and white background noise (65 dB) for 3 min. During block 1, 30 startle trials (105 dB white noise, 20 ms duration) were delivered at 20 s intervals. Block 2 consisted of the presentation of seven different trials presented 10 times each in a pseudo-randomized order and at 30 s intervals: 10 pulse-alone trials and 10 of each of six different prepulse–pulse trial types with three different interstimulus intervals (12, 50 and 100 ms) and two different prepulse sound pressure levels (75 and 85 dB). 'Pulse-alone' trials consisted of startle stimulus only presentation, while 'prepulse–pulse' trials consisted of the presentation of a weaker non-startling prepulse (white noise, 4 ms duration, volume as indicated) at a specific interstimulus interval (ISI) before the startling stimulus. All animals underwent the entire protocol once before injections started (pretest). In some rare cases, animals showed extremely low PPI, potentially caused by the preceding surgery (PPI of less than 35% across multiple ISIs) and these animals were excluded from further experiments.

Data analysis

Data was analysed using Microsoft Excel (Version 14.0.6129.5000, Microsoft, USA) and SPSS (v.20.0.0, IBM, USA). All trials were controlled for potential startle responses to the prepulse. Although the startle threshold for some animals is below 85 dB according to the input/output function, animals did not startle to the prepulses, probably because of their shorter duration of the prepulse (4 ms *vs.* 20 ms in the I/O function). The startle amplitude (peak-to-peak) to the 105 dB pulse was analysed for each trial. PPI was calculated from block 2 data using the following formula:

$$\%\text{prepulse inhibition} = [1 - (\text{prepulse} - \text{pulse trial amplitubes})/(\text{pulse alone trial amplitude})] \times 100$$

Drug effects on PPI were calculated by averaging over the 10 trials of a given stimulus condition. Drug effects on baseline startle amplitude were calculated by averaging the first 20 startle responses in block 1.

In very rare cases, the baseline startle response in an animal was very low and close to the noise level. In this case no prepulse inhibition can be observed, and our methods lead to prepulse inhibition values of below zero (indicating sensitization rather than prepulse inhibition). The respective data points were excluded from analysis. Drug effects were assessed with repeated measurement ANOVAs with drug, prepulse level and inter-stimulus interval as within subject factors. However, for the intracranial injections of MK-801 (see Fig. 2) and ifenprodil (see Fig. 4) the number of subjects with a complete data set with all drug concentration is relatively low. To avoid losing the complete data sets of the respective animals we used a mixed model approach. We ran a general linear model (PROC GLM) with all interactions usually seen in a repeated measure ANOVA and with restricted maximum likelihood as an estimate. Degrees of freedom were calculated with the Kenward–Rogers method. Additional to the within-subject effects (drug, prepulse level and interstimulus interval), the injection site (dorso-caudal and ventro-rostral) was considered as a between-subject effect for these two data sets. Statistics were run in consultation with the LW Stitt Statistical Services (Canada).

Group comparisons and *post-hoc* tests were done using the Bonferroni correction to account for repeated testing. Differences were considered statistically significant when p-values were smaller than 0.05. In the figures data is presented as means (±S.E.M. between animals). Significant differences in means are shown with * for $p<0.05$.

Histology

After completion of testing, animals were euthanized. Intracranial injection of 0.2 μl stock thionine solution was performed post-mortem in order to label injector tips placements, and the brains were removed and fixed in 10% formalin. Brains were then immersed in 10% formalin +15% sucrose for cryoprotection and sectioned, using a freezing microtome, at a thickness of 50 μm. Slices were mounted and stained with thionine, cover slipped and examined for cannula placement. Cannula placement confirmation was performed using a rat brain atlas (Paxinos and Watson, 2005).

Results

In order to test the effects of drugs on PPI, we used two different levels of prepulses, 75 and 85 dB, and three different interstimulus intervals (ISI) between prepulse and startle pulse, 12, 50 and 100 ms. The higher prepulse intensity leads to maximum PPI at an ISI of 50 ms, however, PPI induced by lower prepulse intensities is often more sensitive to disruptions. Furthermore, it is

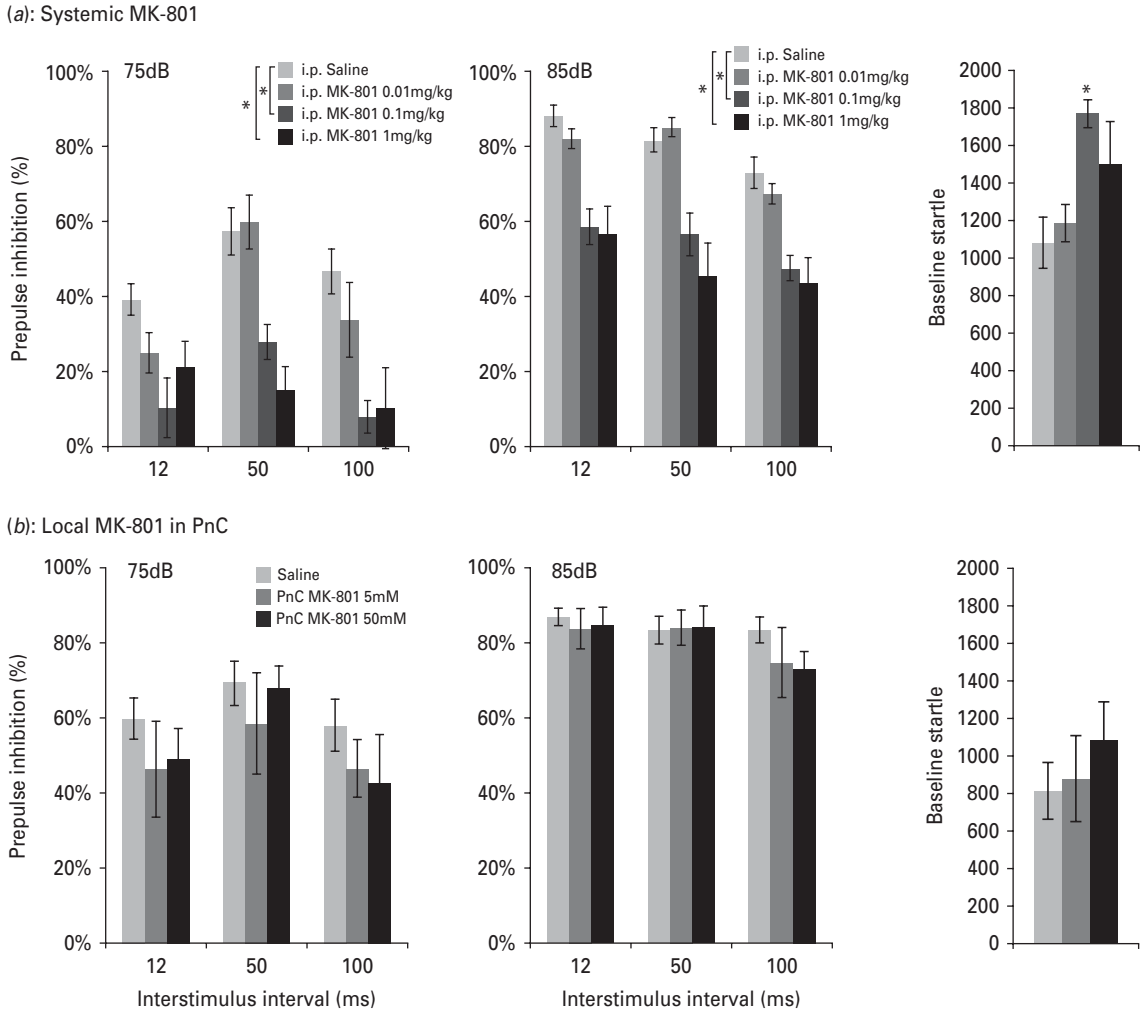

Fig. 1. Systemic MK801, but not local microinfusion into the PnC, disrupt PPI. (*a*) Systemic MK-801 administration dose-dependently disrupts PPI at both 75 and 85 dB prepulse amplitudes (n=7). Systemic MK-801 also significantly increases baseline startle amplitude at the 0.1 mg/kg dose (right panel). Startle amplitudes are displayed in arbitrary units. (*b*) Microinfusion of MK-801 into the PnC had no significant impact on PPI at 75 or 85 dB prepulse levels (n=8). Local microinfusion into the PnC had no significant effect on baseline startle (right panel).

hypothesized that PPI is mediated by different neurotransmitters and receptors at different ISIs (Jones and Shannon, 2000; Yeomans et al., 2010), and is, therefore, differentially susceptible to pharmacological disruptions (see also Mansbach and Geyer, 1991).

Systemic NMDAR antagonists

First, we aimed to reproduce the systemic effect of the NMDAR antagonist MK-801on PPI.

We injected seven animals with three different doses of MK-801 (0.01, 0.1, 1.0 mg/kg), and saline as a control in a pseudorandomized order and at least 5 d apart (Fig. 1(*a*)). As expected, the repeated measurement ANOVA (drug×level of prepulse×ISI) yielded main effects for the level ($F_{1,6}$=294.949, p<0.001) and the ISI ($F_{2,12}$=6.727, p=0.011), as well as a level×ISI interaction ($F_{2,12}$=7.386, p=0.008). More importantly, it also confirmed the drug effect ($F_{3,18}$=29.276, p<0.001). Pairwise comparison (Bonferroni corrected) revealed that both the 0.1 and 1.0 mg/kg doses significantly disrupted PPI, while no significant disruption was observed at the 0.01 mg/kg dose. MK-801 also significantly increased baseline startle amplitude (repeated measurement ANOVA, $F_{3,18}$=6.491, p=0.004). But pairwise comparison showed that only the 0.1 mg/kg dose significantly increased baseline startle amplitude.

In the following experiments we injected MK-801 locally into two different brain regions implicated in mediating/modulating PPI: the PnC, where the startle mediating neurons receive sensory glutamatergic input and modulatory input from different brain regions; and the mPFC which has been previously implicated as an important structure in NMDAR antagonist induced PPI deficits.

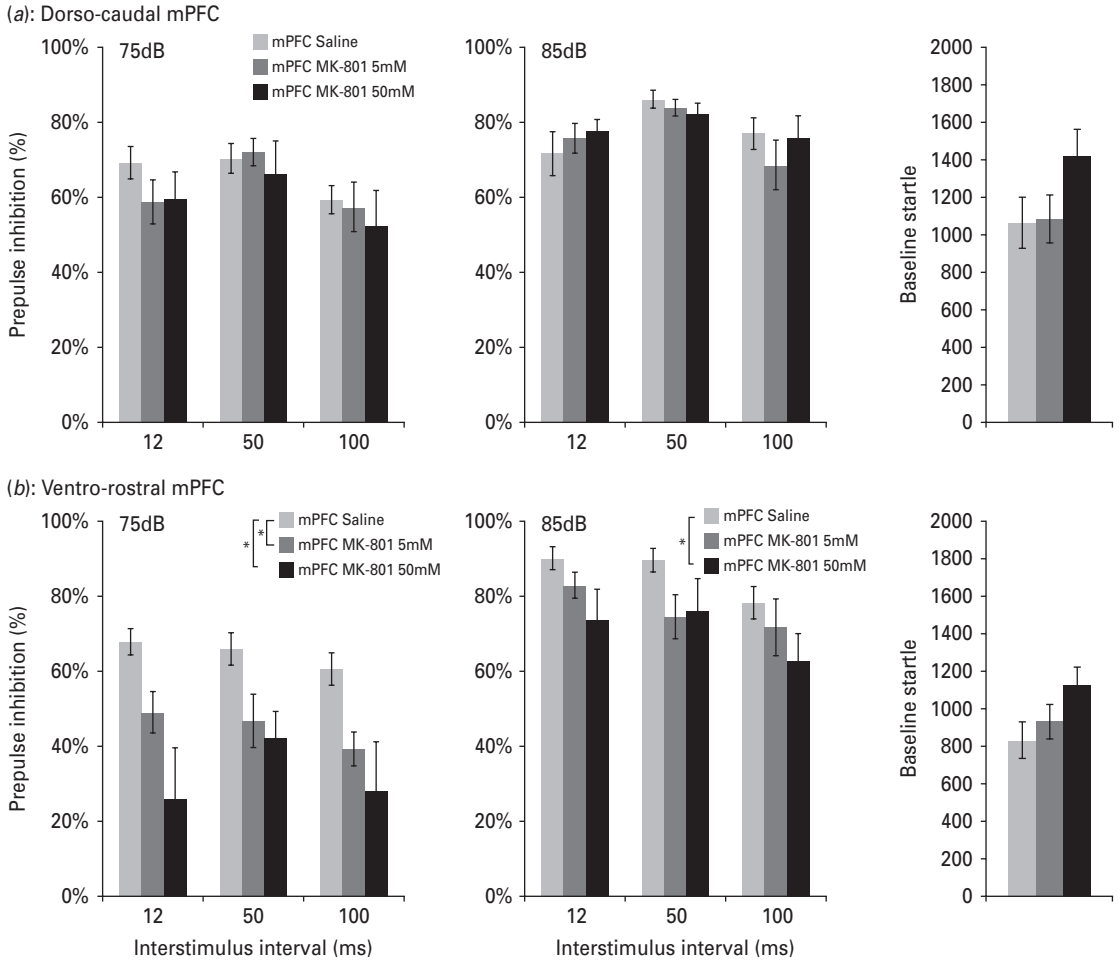

Fig. 2. MK-801 microinfusions into the ventral mPFC disrupts PPI. (*a*) Prepulse inhibition levels after intracranial (i.c.) administration of saline, and two different doses of MK-801, into the dorsal-third and most caudal areas of the mPFC. No significant effect on PPI is observed. Prepulse inhibition values are shown with 75 dB (saline: $n=14$; 5 mM MK-801: $n=14$; 50 mM MK-801: $n=8$) and 85 dB prepulse levels (saline: $n=8$; 5 mM MK-801: $n=8$; 50 mM MK-801: $n=8$). No significant effect on baseline startle amplitude was observed after i.c. mircoinfusions of MK-801 into the dorso-caudal mPFC (right panel). (*b*) Prepulse inhibition levels after i.c. administration of MK-801 to the ventral two thirds and most caudal areas of the mPFC are disrupted at the 75 dB (saline: $n=15$; 5 mM MK-801: $n=15$; 50 mM MK-801: $n=15$) and the 85 dB prepulse (saline: $n=5$; 5 mM MK-801: $n=5$; 50 mM MK-801: $n=5$). Baseline startle was not affected (right panel).

Local injections into the PnC

Eight animals received 5 and 50 mM local MK-801 injections into the PnC, as well as a local saline injection as its own control (each 0.5 μl/side), in a pseudorandomized order and at least 5 d apart (Fig. 1(*b*)). The repeated measurement ANOVA (drug × level of prepulse × ISI) revealed no drug effect ($F_{2,14}=1.3030$, $p=0.382$), nor any interaction of level and ISI with the drug. However, the expected main effects for the prepulse level ($F_{1,7}=19.202$, $p=0.003$) and the ISI ($F_{2,14}=8.000$, $p=0.005$) can still be observed. MK-801 injections into the PnC also had no effect on the baseline startle amplitudes (repeated measurement ANOVA, $F_{2,14}=1.140$, $p=0.348$). Please see supplementary material, Fig. S1 for localizations of PnC cannulae.

Local injections into the mPFC

All animals received one (5 mM, $n=16$) or two (5 and 50 mM, $n=13$) local MK-801 injections and a local saline injection as control in a pseudorandomized order and at least 5 d apart (Fig. 2). Local injections of MK-801 into the mPFC initially revealed very inconsistent effects on PPI: in a first batch of animals PPI was clearly disrupted, whereas in a second cohort PPI was unaffected or even enhanced. Upon further histological analysis of injection sites, a clear image emerged: local injections of MK-801 in the rostral and ventral region of the mPFC led to a disruption of PPI whereas MK-801 injections into the dorsal and caudal parts of the mPFC at the second cohort had no effect, or enhanced PPI. We therefore targeted either the ventro-rostral or the dorso-caudal portion of the mPFC

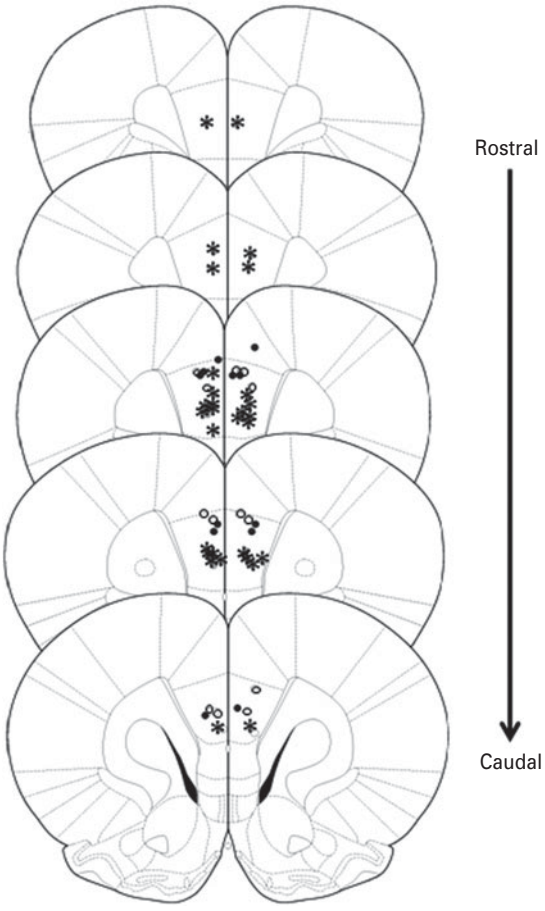

Fig. 3. Localizations of the mPFC microinjections. Coronal sections indicating the location of injector tips in the mPFC, and the local effect of MK-801 injection on PPI at each site. Each asterisk and circle represents injector tip placement in a different animal (animals received bilateral cannulation and injections). Asterisks indicate cannula localizations that lead to a decrease in PPI after injections of MK-801, black circles indicate an increase in PPI, unfilled circles indicate no effect on PPI. Coronal sections shown here are 0.24 mm apart, and the rostral-most section is 3.72 mm anterior to bregma. Atlas illustrations adapted from the atlas of Paxinos and Watson (2005).

in subsequent experiments by adjusting the stereotaxic parameters by 0.5 mm in the respective dimension.

The mixed model (injection site × drug × prepulse level × ISI, BIC=−140.3) on PPI in these animals confirmed this phenomenon. There was a significant main effect for the injected drug ($F_{2,273}$=17.68, p<0.001), as well as an interaction between the injection site and the drug ($F_{2,273}$=8.99, p<0.001). *Post-hoc* analyses indicate that both doses of MK-801 significantly disrupted PPI (5 mM: p=0.006 and 50 mM: p<0.001). However, follow-up analyses for the injection sites at the different prepulse levels showed that injections of MK-801 located approximately within the dorsal third of the mPFC, and within the caudal most 0.5 mm of the rostro-caudal extent of the mPFC, had no effect on PPI with either 75 db ($F_{2,99}$=1.441, p=0.224, n_{Saline}=14, $n_{MK\text{-}801\ 5\ mM}$=14, $n_{MK\text{-}801\ 50\ mM}$=8) or 85 dB prepulse amplitudes ($F_{2,63}$=0.149, p=0.862, n_{Saline}=8, $n_{MK\text{-}801\ 5\ mM}$=8, $n_{MK\text{-}801\ 50\ mM}$=8). In contrast, injections of MK-801 placed within the ventral two-thirds of the mPFC, over most of the rostro-caudal extent of the mPFC except the caudal-most area, significantly disrupted PPI at both, the 75 dB ($F_{2,96}$=18.494, p<0.001, n_{Saline}=15, $n_{MK\text{-}801\ 5\ mM}$=15, $n_{MK\text{-}801\ 50\ mM}$=15) and the 85 dB prepulse amplitude ($F_{2,36}$=4.790, p=0.014, n_{Saline}=5, $n_{MK\text{-}801\ 5\ mM}$=5, $n_{MK\text{-}801\ 50\ mM}$=5) at all ISIs.

There was no significant effect of MK-801 on baseline startle (two-way ANOVA, injection site × drug, drug: $F_{2,65}$=2.532, p=0.087, injections site: $F_{1,65}$=2.706, p=0.105, interaction: $F_{2,65}$=0.152, p=0.859). The exact placements of the injector tips of both groups are displayed in Fig. 3.

In order to evaluate the possible involvement of the NR2B subunit in this effect, we repeated the same microinjections with the non-competitive NMDAR subunit NR2B antagonist ifenprodil (Fig. 4). Animals received either one (1 mM, n=15) or two (100 and 1 mM, n=17) injections of ifenprodil into the mPFC. Those injections did show a similar relationship between cannula placement and drug effect as the MK-801 injections. Though the mixed model analysis (injection site × drug × prepulse level × ISI, BIC=−241.3) on PPI in these animals did not report a significant effect for the injection site ($F_{1,31.2}$= 2.01, p=0.167), or the drug ($F_{2,322}$=2.58, p=0.077) alone, it revealed an interaction between both ($F_{2,322}$= 4.37, p=0.013), as well as between the drug and the prepulse level ($F_{2,322}$=5.01, p=0.007).The follow-up analyses (drug × ISI) for the injection sites at the different prepulse levels depicted a significant effect of the ifenprodil only for the 75 dB prepulse level in the ventro-rostral injections ($F_{2,90}$=5.750, p=0.004, n_{Saline}=15, $n_{Ifen100\ \mu M}$=5, $n_{Ifen1\ mM}$=14) with only the high dose significantly impairing PPI (100 μM: p=0.999, 1 mM: p=0.008). The baseline startle was not significantly affected by the ifenprodil injections (two-way ANOVA, injection site × drug, drug: $F_{2,75}$=0.562, p=0.572, injections site: $F_{1,75}$=1.705, p=0.196, interaction: $F_{2,75}$=1.335, p=0.269).

NMDA antagonist combined with LY354740

It has previously been shown that activation of mGluR2/3 in the mPFC inhibits glutamate release and thereby opposes the effect of NMDAR antagonists that are assumed to increase glutamate release in the mPFC (see Introduction). We therefore tested whether the injection of LY 354740 into the ventro-rostral mPFC (10 mM, 0.5 μl/side) reverses PPI disruption caused by MK-801 (0.1 mg/kg) (Fig. 5). Ten animals were tested on three different days with a recovery period of at least 5 d in between. They have been tested after the following treatments: (1) systemic and local saline, (2) systemic MK-801 and local saline and (3) systemic MK-801 and local LY 354740. The order of the treatments was pseudorandomized.

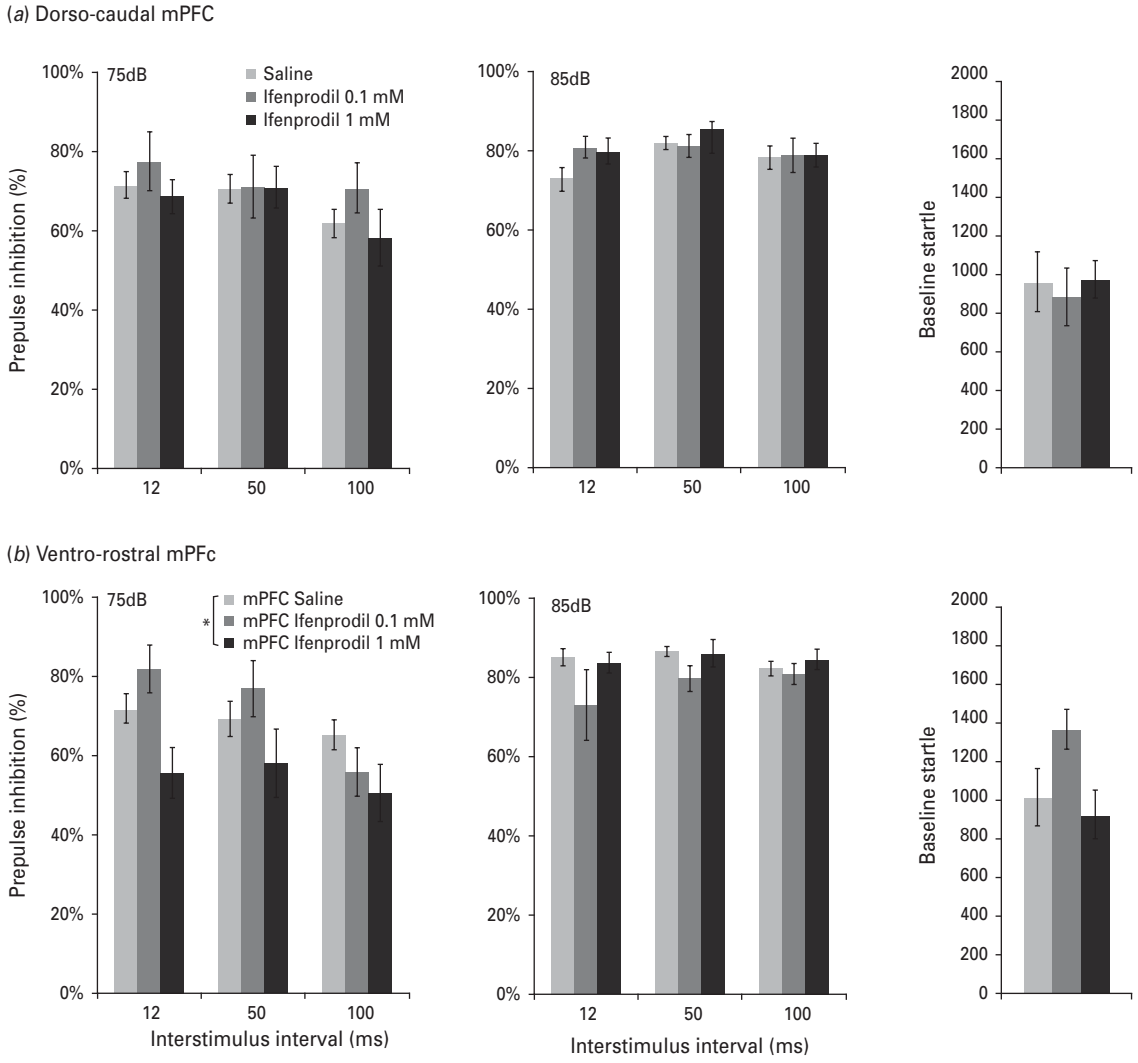

Fig. 4. Microinfusions of ifenprodil into the ventral mPFC disrupts PPI. (*a*) Prepulse inhibition levels after intracranial (I.C.) administration of the NR2B specific NMDA receptor antagonist ifenprodil into the dorsal-third and most caudal areas of the mPFC. Rats received bilateral i.c. injections of 0.9% saline (control, 0.5 μl/side) and ifenprodil (100 and 1 mM, 0.5 μl/side). Prepulse inhibition at 75 dB (saline: n=17; 100 μM ifenprodil: n=12; 1 mM ifenprodil: n=17) and 85 dB prepulse levels (saline: n=12; 100 μM ifenprodil: n=12; 1 mM ifenprodil, n=12) was not significantly altered. Baseline startle amplitudes were also not affected (right panel). (*b*) Prepulse inhibition levels after i.c. administration of ifenprodil to the ventral two thirds and most caudal areas of the mPFC are disrupted. No effect on baseline startle amplitude was observed (right panel). PPI was disrupted at the 75 dB prepulse level (saline: n=15; 100 μM ifenprodil: n=4; 1 mM ifenprodil: n=14), but not the 85 dB prepulse level (saline: n=5; 100 μM ifenprodil: n=5; 1 mM ifenprodil: n=5).

A repeated measurement ANOVA (drug × prepulse level × ISI) reported a significant drug effect ($F_{2,18}$= 31.645, p<0.001), as well as a drug × level interaction ($F_{2,18}$=3.770, p=0.043) next to the expected level and ISI effect. Pairwise comparison confirmed previously described effect off MK-801 (p<0.001, compare Fig. 1(*a*)), but also showed that local LY 354740 injections recovered PPI (p=0.008). However the local LY 354740 injections are still significantly different to the saline/saline controls (p=0.005), indicating only a partial recovery. Follow-up ANOVAs for the two different prepulse levels showed that this recovery is only significant at the 85 dB prepulse, but there is also a trend for a recovery at the 75 dB prepulse. The baseline startle amplitude was not affected by the drugs (repeated measurement ANOVA, $F_{2,18}$= 1.659, p=0.218).

Discussion

Our results confirm a robust disruption of PPI by systemic administration of MK-801, and an increase of baseline startle, according to previous studies (Mansbach and Geyer, 1989; Chaperon et al., 2003). Interestingly, local application of MK-801 into the PnC did not affect PPI or baseline startle, indicating that NMDARs expressed in the PnC play only a minor role in these behavioural

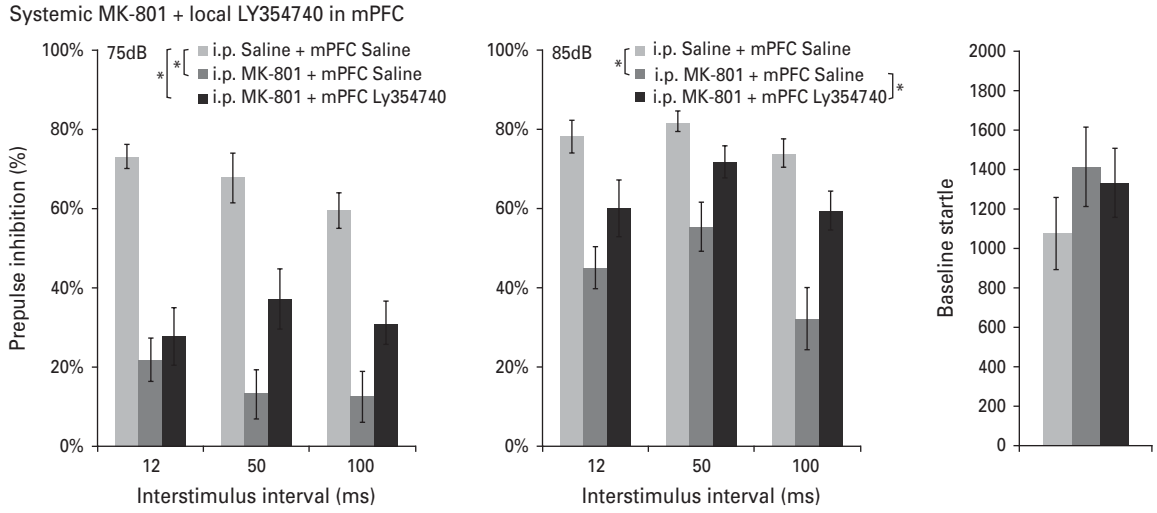

Fig. 5. Local microinfusion of LY354740 reverses systemic MK-801 effects. Prepulse inhibition levels after combined systemic administration of MK-801 (0.1 mg/kg) and local microinjection of either saline or LY354740 (500 μM) into the ventral mPFC (n=10). Local injections of LY354740 significantly reversed the effect of systemic MK-801 at the 85 dB prepulse level. The baseline startle amplitude was not affected by the injections (right panel).

responses. In contrast, local MK-801 application in the mPFC disrupted PPI, at least at the 75 dB prepulse levels, indicating a major role of NMDARs in the mPFC in mediating the PPI disruptions induced by systemic NMDAR antagonists. The baseline startle response was not affected. The effect of local MK-801 application on PPI was markedly smaller than that of systemic MK-801 application, and limited to the more vulnerable 75 dB prepulse paradigm. This could be due to the fact that local drug applications might not be effective over the entire mPFC area important for PPI modulation, due to the increasing dilution of the drug as it diffuses away from the injection site. Alternatively, brain areas other than the mPFC might contribute to the systemic effect of MK-801, and these might also be responsible for the MK-801 effect on baseline startle responses. Local application of LY 354740 into the mPFC paired with systemic MK-801 application rescued PPI only partially. This again, could be due to either spatially limited effects of local drug injections into the mPFC, or the additional contribution of NMDARs outside the mPFC. A strong argument for the first explanation lies in the fact that systemically induced PPI deficits could be eliminated by prior lesions to the mPFC (Schwabe and Koch, 2004). In any case, NMDARs in the mPFC play a major role in these systemically induced PPI disruptions, and our data indicate that this NMDAR population includes NR2B subunit containing receptors, since local MK-801 effects could be mimicked by the local injection of ifenprodil.

MK-801 microinfusion to the mPFC has been previously shown to cause only trend-level inhibition of PPI (Bakshi and Geyer, 1998). Our results showed a relationship between injection location within the mPFC and the effect of NMDA antagonism. Our injections were targeted to the prelimbic area of the mPFC. Only injections to the ventral two-thirds of the prelimbic area were effective in disrupting PPI. Injections to the dorsal third, and the most caudal 0.5 mm of the mPFC had no effect on PPI. This division between the dorsal and ventral injection sites with respect to the effect of NMDA antagonism within the prelimbic cortex of rats may explain the inconclusive effect of local NMDA antagonism found by others.

Our results support the notion that there exists a division of the medial prefrontal cortex into a dorsal and a ventral component, and only NMDARs in the ventral portion appear to play a role in PPI modulation. On the basis of functional and anatomical criteria it has been previously suggested that there exists a dorsal compartment within the medial prefrontal cortex encompassing the FR2, dorsal anterior cingulate areas, and the dorsal part of the prelimbic area; and a ventral compartment that includes the ventral prelimbic, infralimbic and medial orbital areas (Gisquet-Verrier et al., 2000, for review see Heidbreder and Groenewegen, 2003; Rogazzino, 2007). This distinction is associated with differences in cytoarchitecture, connectivity patterns, neurochemistry and immediate early genes expression. Functionally, it appears that the dorsal regions are involved with generating rules associated with temporal ordering and motor sequencing of behaviour (see reviews Gisquet-Verrier et al., 2000; Kesner, 2000), or behavioural flexibility when conditions require a shift in strategy (Uylings et al., 2003), whereas the ventral regions are involved in attentional and response selection functions as well as visual working memory (e.g. Granon and Poucet, 2000). Our results support a functional distinction between ventral and dorsal mPFC, and suggest that only the ventral part of the mPFC modulates PPI, which is interesting in

light of the fact that PPI and attentional disruptions seem to be associated.

A study by Sullivan and Gratton (2002) showed that lesioning the ventral part of the mPFC (ventral prelimbic plus infralimbic cortex) does not affect PPI. This is in accordance with many previous studies that demonstrated that the mPFC is not important for normal PPI, but rather plays a modulatory role (for review see Yeomans et al., 2006), although Yee (1999) found a modest reduction of PPI by large mPFC lesions. In our study, NMDA antagonists were applied to this region, presumably leading to a higher activity of pyramidal neurons in the ventral mPFC, according to the studies of Mogaddam et al. (1997) and others (see Introduction). Taken together, these results indicate that the activation of the ventral mPFC leads to a disruption of PPI, whereas the inactivation of this region does not impact PPI.

Conclusion

In summary, this study confirms that NMDARs receptors in the mPFC, but not within the brainstem structures of the primary startle pathway, play an important role in modulating sensorimotor gating. The results further implicate that NR2B subunit containing receptors at least partially mediate this effect. Most importantly, only the inhibition of NMDA receptors expressed in the ventral portion of the mPFC are able to disrupt PPI, whereas NMDA antagonism in the dorsal region had no, or an opposite, effect. These findings emphasize the notion that there are functional differences between the dorsal and ventral regions of the mPFC, and more specifically between the dorsal and the ventral portions of the prelimbic area.

Acknowledgments

This work was supported by the Ontario Mental Health Foundation (OMHF), the Natural Sciences and Engineering Research Council of Canada (NSERC), and the Canadian Institute for Health Research (CIHR)

Statement of Interests

None.

Supplementary material

For supplementary material accompanying this paper, visit http://dx.doi.org/10.1017/S1461145713001041

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