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Clozapine enhances disruption of prepulse inhibition after sub-chronic dizocilpine- or phencyclidine-treatment in Wistar rats

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Abstract

Sensitisation (i.e. progressive enhancement) of behavioural abnormalities induced by repeated treatment with non-competitive NMDA receptor antagonists in animals is considered an animal model for schizophrenia. Here, male Wistar rats were treated for 11 days with either dizocilpine (0.1 mg/kg), phencyclidine (PCP, 2 mg/kg), or saline and tested for prepulse inhibition (PPI) of the acoustic startle response (ASR). The aims of this study were twofold: First, we tested whether sensitisation of PPI deficits previously found in Sprague–Dawley rats were also found in Wistar rats, and, second, whether these effects can be ameliorated by the atypical antipsychotic clozapine. PPI is a paradigm for the assessment of sensorimotor gating (and its deficits) and is impaired in schizophrenic patients. After the sub-chronic treatment the rats were tested drug-free (day 12), and on the following days after drug challenge by PCP (2 mg/kg), combinations of PCP (2 mg/kg) and clozapine (5 and 10 mg/kg), or clozapine (5 mg/kg) alone. PPI was significantly reduced by both NMDA receptor antagonists. This effect was not further enhanced by the daily treatment. Startle magnitude was increased after eight days of dizocilpine-treatment only, indicating sensitisation of startle-potentiation by this drug. Testing the rats drug-free on day 12 revealed enhanced PPI and reduced startle (compared to the matching test on day 0) irrespective of previous treatment. Drug challenge with PCP (2 mg/kg) again reduced PPI in all groups. Clozapine (5 and 10 mg/kg) failed to antagonise the PPI-disruptive effects of PCP and even *enhanced* the PCP-induced PPI-deficits in rats pretreated with PCP or dizocilpine. These findings suggest: (1) that PPI and startle are influenced differently by non-competitive NMDA receptor antagonists, (2) that PCP and dizocilpine reduce PPI in Wistar rats, but do not lead to a sensitisation of this effect; and (3) that under the present schedule of treatments, the antipsychotic compound clozapine does not antagonise but rather enhances PPI-disruptive effects of non-competitive NMDA receptor antagonists, pointing towards a complex interaction of the brain processes underlying the action of psychotomimetic and atypical antipsychotic drugs.

Keywords: Acoustic startle response; Antipsychotic; MK-801; NMDA-receptor; Schizophrenia; Sensitisation; Sensorimotor gating

1. Introduction

Long-term exposure to non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonists such as phencyclidine (PCP) or the prototypical antagonist dizocilpine (also known as MK-801) in laboratory animals induces a variety of physiological and behavioural consequences that have been considered to model certain aspects of neuropsychiatric disorders such as schizophrenia (Jentsch et al., 1997; Jentsch and Roth, 1999; Konradi and Heckers, 2003).

Prepulse inhibition (PPI) of the acoustic startle response (ASR) is a simple behavioural measure of sensorimotor gating that is observed when the startling noise pulse is shortly (30–800 ms) preceded by a weaker sensory prepulse (Hoffman and Ison, 1980; Koch, 1999). PPI is reduced in schizophrenic patients (and in their healthy relatives) and in some other neuropsychiatric disorders (Braff et al., 1978, 2001; Weike et al., 2000). Experimentally reduced PPI has been widely used in animals as a model of PPI-deficits (and their potential neuropathological correlates) in schizophrenia (Braff et al., 2001;

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Geyer et al., 2001; Koch and Fendt, 2003; Koch and Robbins, 2001; Swerdlow et al., 2001). Psychopharmacological studies in rodents have shown a particularly high degree of predictive validity for PPI as a behavioural tool to assess the antipsychotic potential of drugs. PPI-deficits induced by dopamine agonists are reversed by both typical and atypical antipsychotics, while PPI-deficits induced by serotonin agonists or NMDA receptor antagonists are only attenuated or reversed by atypical antipsychotics, such as clozapine (Geyer et al., 2001). However, *prolonged* treatment of rats with the typical antipsychotic compound haloperidol (a preferential dopamine D2 receptor antagonist) has been shown to attenuate a PCP-induced PPI-deficit (Martinez et al., 2000).

In addition to the acute treatment of rodents with PCP or related compounds, the effects of chronic or sub-chronic application of this psychotomimetic drug on rodent behaviour–including PPI–has also been investigated (Jentsch and Roth, 1999). Interestingly, PPI in rats was disrupted during, but not after chronic, PCP-treatment in rats (Martinez et al., 1999), despite the fact that long-lasting changes in brain neurochemistry are induced by sustained treatment with PCP e.g. impairment of prefrontal dopaminergic activity (Jentsch et al., 1998), and reduced $GABA_A$-receptor-mediated inhibition in the septum (Yu et al., 2002).

In a recent study a repeated daily treatment of Sprague–Dawley rats for 11 days with the PCP-site agonist dizocilpine, which is 2–10 times more potent than PCP as NMDA receptor antagonist (Koek et al., 1988), induced a PPI-deficit that increased in magnitude during the course of treatment (Schulz et al., 2001). This kind of sensitisation of a behavioural response, after chronic treatment with psychotomimetic drugs, has been considered as particularly relevant to the development of psychoses (Jentsch and Roth, 1999; Wolf, 1998). Sensitisation of drug effects (especially by using chronic PCP-treatment) on animal behavior has also been considered as a model for the worsening of symptoms in schizophrenia (Jentsch et al., 1998; Jentsch and Roth, 1999; Lieberman et al., 1997).

We here attempted to translate our previous findings in rats of the Sprague–Dawley strain onto Wistar rats, which have already been shown to be less sensitive to the PPI-disruptive effects of dopamine agonists and NMDA receptor antagonists (Varty and Higgins, 1994). Strain- and breeder-differences are an important issue in PPI research since they offer the possibility to investigate genetic trait differences in the expression of sensorimotor gating rather than acute or environmental differences (Swerdlow et al., 2000, 2004; van den Buuse, 2003).

The present study had three main objectives: (1) to replicate our previous finding of sensitisation of a PPI-deficit in Sprague–Dawley rats by repeated treatment with dizocilpine in rats of the Wistar strain, (2) to test whether PCP has similar effects as dizocilpine on PPI, and (3) to test whether the effects of dizocilpine and PCP on PPI can be reversed by the atypical antipsychotic drug clozapine.

2. Material and methods

2.1. Animals

Twenty-eight adult male Wistar rats (Harlan Winkelmann, Borchen, Germany) were used in this study. They were kept in groups of seven in standard cages (Macrolon type IV, 60×38×25 cm^3) under controlled ambient conditions (temperature: 22 °C, humidity: 55%) under a continuous 12-h light–dark cycle (lights on at 07:00 h). They received tap water ad libitum and rat chow (12 g/animal/day; Nohrlin 10H10, Bad Salzuflen, Germany), in order to maintain a body weight of 250–300 g during testing. Testing was done between 12:00–17:00 h. All experiments were performed in strict adherence to national laws and international recommendations for the use and care of experimental animals (European Communities Council Directive of 24/11/86; 86/609/EEC).

2.2. Experimental procedure

The experiment consisted of four blocks: (1) Matching of three groups for similar baseline of ASR and PPI; (2) Daily drug-treatment and PPI-test for 11 days ("Pretreatment": PCP N=10, dizocilpine N=9, saline N=9); (3) First drug-free PPI-test on day 12; (4) Further tests on days 13–21: Four drug-challenge treatments were given in the following sequential order: PCP alone; PCP+5 mg/kg clozapine; PCP+10 mg/kg clozapine; 5 mg/kg clozapine alone with at least 1-day rest between tests and treatments.

For measuring the ASR and PPI using the "Startle Response System" (TSE, Bad Homburg, Germany), the rats were placed into wire-mesh cages (27×9×10 cm^3) onto a piezo-accelerometer in a sound-attenuated chamber (32×32×32 cm^3). All acoustic stimuli were presented by two loudspeakers mounted in a distance of about 8 cm from the animal. The rats were accustomed to the testing apparatus for 5 min before testing began. For the assessment of PPI, they received 15 trials of 5 different trial types (1. No-Stimulus, 2. Pulse-alone: 20 ms white noise pulse of 100-dB sound pressure level (SPL), 3. Prepulse-alone: 72-dB tone pulse of 10 kHz, 4. and 5. Prepulse–Pulse trials: prepulse of 64 or 72 dB followed after 100 ms by a 100-dB pulse) in a pseudo-random order with an inter-trial interval of 20–30 s, so that a test session lasted about 35 min. Background white noise level was set at 60 dB SPL. ASR magnitudes were expressed as arbitrary units. PPI was calculated as percent score: Pulse alone ASR magnitude minus ASR magnitude in Prepulse+Pulse-trials, divided by the Pulse alone magnitude, multiplied by 100.

2.3. Drugs

Phencyclidine (PCP; (1-[1-Phenylcyclohexyl] piperidine) Hydrochloride; Sigma, Germany) was dissolved in saline (pH 5) and was injected subcutaneously in a dose of 2 mg/

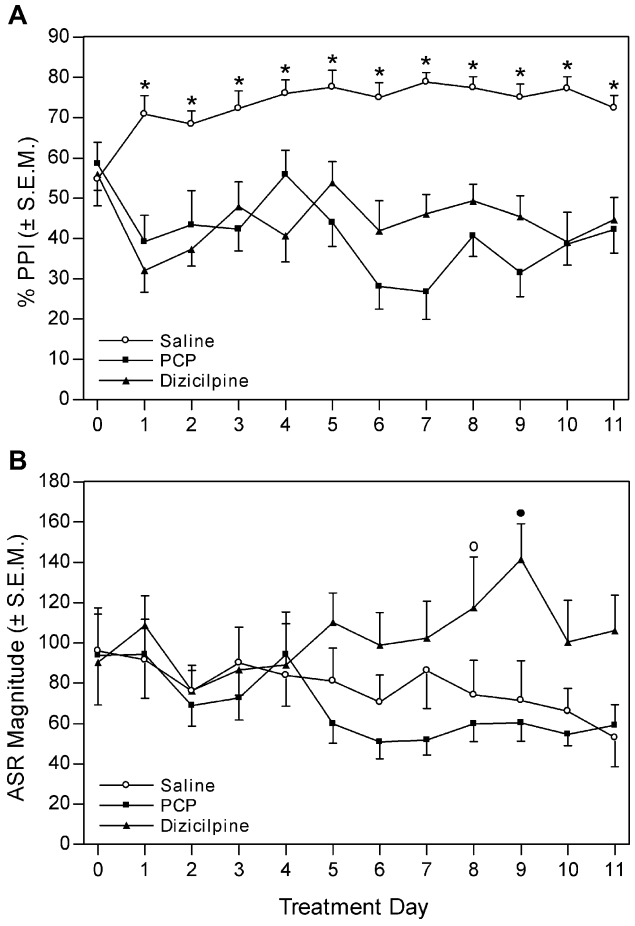

Fig. 1. (A) Mean percent (%) PPI and (B) mean ASR magnitude (+standard error of the mean, S.E.M.) of the 3 experimental groups recorded over 11 days of treatment/testing. Asterisks indicate statistically significant differences between saline-treated rats (N=9) and the PCP- (N=10) as well as dizocilpine- (N=9) treated groups. In panel (B), the open circles indicate significant differences between dizocilpine and PCP, and the filled circles between dizocilpine and PCP or saline (p<0.05 in post hoc Tukey's t-tests computed after significant main effects of ANOVA).

kg 10 min before the test. Dizocilpine (+MK-801, (5*S*,10*R*)-(+)-5-Methyl-10,11-dihydro-5*H*-dibenzo[a,d]cycloheptem-5,10-imine maleate; Sigma, Germany) was dissolved in saline (pH 5) and was injected subcutaneously in a dose of 0.1 mg/kg 15 min before the test. Clozapine (Sigma, Germany) was dissolved in saline (using a drop of 1 N HCl and adjusted to pH 5 with 1 N NaOH) and injected intraperitoneally in doses of 5 and 10 mg/kg 30 min before the test. Saline (pH 5) was used as vehicle control. Injection volumes were 1 ml/kg.

2.4. Data analysis

All statistical analyses were performed with the statistical software Sigma Stat (Version 2.00 for Windows). Mean ASR magnitudes and percent PPI were analysed with analyses of variance (ANOVA) using drug-treatment (pre-treatment and challenge), test day, and prepulse intensities as factors. Post hoc tests were done using Tukey's t-test. Two-tailed levels of significance were used and p<0.05 was considered as significant.

3. Results

Due to the matching procedure on day 0, the 3 treatment-naïve groups of animals (saline, dizocilpine and PCP) did not differ with respect to their mean ASR magnitudes and mean percent (%) PPI-scores. Since there was no significant interaction between prepulse-intensity and treatment effects (two-way ANOVA: factor *prepulse*, p<0.001; factor *treatment*, p<0.001; interaction between prepulse and treatment, p=0.82), PPI-scores on 64-dB- and 72-dB trials were pooled for all further analyses. Both PCP and dizocilpine induced a PPI-deficit on the first day of treatment/testing (Fig. 1A), while no effect on the ASR magnitude in pulse-alone trials was observed (Fig. 1B). Percent PPI increased in saline-treated rats during the first few days and remained on a stable score of about 75% during the 11-day test period. In contrast, both treatments with 2 mg/kg PCP and 0.1 mg/kg dizocilpine reduced PPI throughout the whole test period. A two-way ANOVA showed a significant effect for the factor *treatment* ($F_{2,335}$=17.492, p<0.001) but not for the factor *day* ($F_{11,335}$=1.339, p=0.203). The interaction between these factors was significant ($F_{22,335}$=2.357, p<0.001). Post hoc tests revealed that PPI was significantly lower in PCP and dizocilpine-treated rats compared to saline-treated rats on all training days (Tukey's t-test, p<0.05). However, as shown in Fig. 1A, there was no consistent enhancement of the PPI-deficit by repeated drug-treatment across the test period of 11 days. The ASR magnitude in pulse-alone trials slightly decreased in saline- and PCP-treated rats, but was enhanced by dizocilpine. A two-way ANOVA showed a significant effect for the factor *day* ($F_{11,335}$=1.864, p=0.044) but not for the factor *treatment* ($F_{2,335}$=2.218, p=0.130) while the interaction between these factors was significant ($F_{22,335}$=2.054, p=0.004). Post hoc testing revealed that, at treatment day 8, the ASR magnitude was significantly higher in dizocilpine-treated rats compared to PCP-treated rats, while at day 9 the ASR was significantly higher in dizocilpine-treated rats compared to PCP- and saline-treated rats (Tukey's t-test, p<0.05).

Table 1
Mean percent (%) PPI (±standard error of the mean, S.E.M.) of the 3 experimental groups tested drug-free before and after chronic PCP-, dizocilpine- or saline-pretreatment

Treatment	Day 0 (Matching)	Day12 (drug-free)
Saline pretreatment	54.70±6.62	72.05±3.40*
PCP pretreatment	58.57±5.30	69.94±3.34*
Dizocilpine pretreatment	55.88±3.95	75.25±3.48*

* Statistically significant differences between day 0 and day 12 (p<0.05 in post hoc Tukey's t-tests computed after significant main effects of ANOVA).

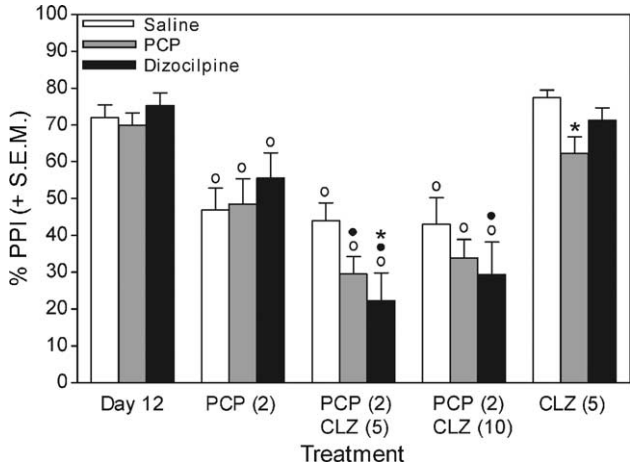

Fig. 2. Mean percent (%) PPI (+standard error of the mean, S.E.M.) of the 3 experimental groups tested drug-free on day 12 after chronic PCP-, dizocilpine-, or saline-pretreatment, as well as after challenge with PCP [2 mg/kg, PCP (2)] and clozapine [5 and 10 mg/kg, CLZ (5) or (10)] on days 13–21. Statistically significant group differences are shown as asterisks (saline-pretreated versus dizocilpine- or PCP-pretreated rats), open circles (day 12 versus respective test day within pre-treatment-groups), filled circles (PCP-challenge versus respective test day within pre-treatment-groups). $P<0.05$ post hoc Tukey's *t*-tests computed after significant main effects of ANOVA.

On day 12, all groups were tested drug-free for ASR and PPI. Compared to the matching test on day 0, all groups showed an enhancement of PPI and a reduction of the ASR magnitude (in pulse-alone trials) irrespective of drug-treatment. For PPI, a two-way ANOVA revealed a significant effect for the factor *day* ($F_{1,55}$=25.863, $p<0.001$) but not for the factor *treatment* ($F_{2,55}$=0.0533, p=0.946) and not for the interaction between these factors ($F_{2,55}$=0.599, p=0.557). Post hoc testing showed that PPI was increased on day 12 compared to day 0 in all groups (Tukey's *t*-test, $p<0.05$; Table 1). For ASR, a two-way ANOVA revealed a significant effect for the factor *day* ($F_{1,55}$=10.927, p=0.003) but not for the factor *treatment* ($F_{2,55}$=0.0325, p=0.968) and not for the interaction between these factors ($F_{2,55}$=0.326, p=0.725). Post hoc testing showed that ASR was reduced on day 12 compared to day 0 for PCP-treated rats (Tukey's *t*-test, $p<0.05$; data not shown). On days 13–21, all 3 groups received a challenge treatment with PCP (2 mg/kg), PCP (2 mg/kg)+clozapine (5 and 10 mg/kg), or clozapine (5 mg/kg) alone. PCP induced a PPI-deficit in all groups that was not dependent upon the pretreatment (Fig. 2). However, there was no antagonistic effect of clozapine on the PCP-induced PPI-deficit in saline-pretreated rats, and, moreover, there was an exacerbation by clozapine of the PCP-induced deficits in PPI of PCP- and dizocilpine-pretreated rats. Additionally, clozapine alone reduced PPI in PCP pretreated rats. A two-way ANOVA revealed a significant effect for the factor *pretreatment* ($F_{4,279}$=52.130, $p<0.001$) but not for the factor *treatment* ($F_{2,279}$=1.227, p=0.301). The interaction between these factors were significant ($F_{8,279}$=2.054, p=0.042). Post hoc testing revealed that PCP-treatment alone reduced PPI significantly in all groups compared to no treatment (drug-free) on day 12. Combined treatment with 5 mg clozapine+2 mg PCP further reduced PPI significantly in the PCP- and dizocilpine-pretreated groups compared to 2 mg PCP only and, in case of dizocilpine pretreatment, also compared to saline-pretreated rats (Tukey's *t*-test, $p<0.05$). Combined treatment with 10 mg clozapine+2 mg PCP enhanced the PPI-disruptive effect of 2 mg PCP only in dizocilpine-pretreated rats. Clozapine alone reduced PPI in PCP-pretreated compared to saline-pretreated rats (Student's *t*-test, $p<0.05$; Fig. 2). No effect on ASR magnitude was observed. A two-way ANOVA showed neither significant effects for the factors *pretreatment* ($F_{3,111}$=2.072, $p<0.111$) and *treatment* ($F_{2,111}$=0.670, p=0.521) nor for the interaction between these factors ($F_{6,111}$=0.189, p=0.979; Table 2).

4. Discussion

The present study investigated the effects of repeated administration of the non-competitive NMDA receptor antagonists dizocilpine and PCP on PPI and the ASR magnitude in Wistar rats. We also tested the effects of the atypical antipsychotic compound clozapine on the behavioural effects of dizocilpine and PCP.

Consistent with several previous reports in rats and mice, we found a profound deficit in PPI during acute dizocilpine- or PCP-treatment (Geyer et al., 2001). These effects are probably mediated by a forebrain network consisting of the dorsal hippocampus, the prefrontal cortex, and the amygdala (Bakshi and Geyer, 1998; Fendt et al., 2000; Schwabe and Koch, 2004). However, a role for mesoaccumbal dopamine in the effects described here cannot be ruled out, since PCP and dizocilpine enhance dopamine-release in the nucleus accumbens (Ault and Werling, 1999) and PPI is regulated potently by mesoaccumbal dopamine (Swerdlow et al., 1992).

In contrast to our previous study (Schulz et al., 2001), the PPI-deficit induced by daily dizocilpine- or PCP-treatment

Table 2
Mean startle amplitude (±standard error of the mean, S.E.M.) of the 3 experimental groups tested drug-free on day 12 and after challenge with PCP [2 mg/kg, PCP(2)] and clozapine [5 and 10 mg/kg, CLZ(5) or (10)] on days 13–21

Treatment	Day 12 (drug-free)	PCP (2)	PCP (2) CLZ (5)	PCP (2) CLZ (10)	CLZ (5)
Saline pretreatment	65.50±13.11	67.21±13.78	51.97±6.93	55.90±8.48	57.76±9.54
PCP pretreatment	58.83±9.65	64.56±11.82	54.26±11.69	63.14±9.60	46.81±6.69
Dizocilpine pretreatment	71.62±8.48	81.80±21.94	59.37±8.31	66.53±17.24	66.96±9.78

showed no significant sensitisation. This might be due to strain-differences between Wistar and Sprague–Dawley rats which have been described extensively before, especially with respect to the effects of dopamine receptor agonists on PPI (Varty and Higgins, 1994), but also with respect to other drug-effects on PPI (Swerdlow et al., 1998, 2000). These studies have shown that Sprague–Dawley rats generally appear to be more vulnerable to PPI-disruption by drugs. For example, in the study by Martinez and co-workers, 1.5 mg/kg of PCP reduced PPI to below 10% (Martinez et al., 1999), whilst in our present study Wistar rats treated with 2 mg/kg PCP still showed around 35% PPI. In our previous investigation on the sensitisation of PPI-disruption by dizocilpine in Sprague–Dawley rats, the drug effects were only very moderate on the first treatment and test days, whilst in the present study, both NMDA receptor antagonists produced a pronounced PPI-deficit already upon first administration. Hence, the failure of the present study to detect a sensitisation effect might be due to a floor effect in the sense that the maximal PPI-deficit that can be induced by relatively low doses of PCP and dizocilpine was already reached. However, the fact that the potentiation of the ASR magnitude by dizocilpine shows a further enhancement (at least until day 9 of treatment) indicates that the physiological effects of prolonged exposure to this drug do sensitise.

Interestingly, we found a dissociable effect of PCP and dizocilpine on the ASR magnitude in the absence of prepulses. While animals treated with PCP showed long-term ASR habituation similar to vehicle-treated rats, and as described before (Koch, 1999; Leaton and Supple, 1986), the rats treated with dizocilpine showed an increasing enhancement of the ASR magnitude across treatment and test days. The ASR magnitude can be enhanced in a variety of ways, for example by the loss of inhibition of motorneurons after administration of the glycine receptor antagonist strychnine (Kehne et al., 1981; Koch and Friauf, 1995), but also by negative emotive states such as fear and anxiety (Davis et al., 1991; Koch and Fendt, 2003; Lang et al., 2001). The experimental design used in the present study does not allow to conclude whether any of these explanations apply, or to discern between these possibilities. Since dizocilpine usually has motor stimulatory effects (Mele et al., 1998), one might assume that the enhancement of the ASR seen here could be due to a general enhancement of motor systems, including the primary ASR circuit located in the brainstem (Koch, 1999). However, this is unlikely because locomotor activity and ASR magnitude show an inverse relationship (Wecker and Ison, 1986). Taken together, the sensitisation of the ASR enhancement by dizocilpine, but not by PCP, is a novel finding that cannot yet be explained on the behavioural level, but points to an important pharmacological difference between PCP and its prototypical agonist dizocilpine (Carter, 1995; Iversen, 1994; Wiley et al., 2003).

PPI-deficits were only found after acute treatment with the NMDA receptor antagonists, but not while testing the rats one day after the last of 11 daily injections. At this time-point, blood levels of dizocilpine and PCP were probably very low since the half-life of both compounds in rats is below 8 h (Andiné et al., 1999; Shelnutt et al., 1999). Hence, similar to the findings of a previous study by the Swerdlow group (Martinez et al., 1999), long-term treatment with PCP or dizocilpine has no carry-over effects on PPI, despite other studies showing that PCP (although in a higher dose than used here) leads to long-lasting changes in the meso-prefrontocortical dopamine system (Jentsch et al., 1997, 1998). Instead, a slight but statistically significant improvement of PPI-performance was found in all three groups of animals (Table 1), which might be due to long-term habituation of the ASR.

PPI-deficits induced by various drugs (mainly dopamine agonists, glutamate antagonists and serotonin agonists) may have predictive validity for antipsychotic drug-activity (Geyer et al., 2001). However, in the present study, the PPI-deficit induced by PCP in saline-pretreated rats was not reversed by clozapine. Clozapine is an atypical antipsychotic compound that is widely used for the treatment of psychosis. It is a dibenzodiazepine-derivative with antagonistic effects on dopamine (D2, D4)-, serotonin (5-HT2)-, muscarinic acetylcholine-, histamine-, and α_1-noradrenaline receptors. The lack of extrapyramidal side effects, and, hence, its atypicality might be due to a low affinity for striatal dopamine D2 receptors, and a relatively high affinity to dopamine D4 receptors (Serretti et al., 2004). It has been shown to reverse PPI-deficits induced by both dopamine receptor agonists and glutamate NMDA receptor antagonists, but there were also reports of a lack of effect on these treatments (Geyer et al., 2001). The present failure of a mitigation by clozapine of the PPI-deficit induced by moderate doses of PCP is consistent with some previous studies (Bast et al., 2000; Hoffman et al., 1993), and suggests that in Wistar rats NMDA receptor antagonists disrupts PPI in a way that may model those PPI-deficits seen in schizophrenic patients which are found despite otherwise effective antipsychotic treatment (Hamm et al., 2001) as well as those deficits found in healthy relatives of schizophrenic patients (Cadenhead et al., 2000). Since antipsychotic effects usually emerge after chronic treatment of schizophrenic patients, it is possible that a reversal of PCP- or dizocilpine-induced PPI deficits in rats will be seen after chronic administration of clozapine. This should be tested in further studies.

The most surprising finding of the present study was that the atypical antipsychotic drug clozapine *exacerbated* the PPI-deficits induced by NMDA receptor antagonists in those rats that had been pretreated with PCP and dizocilpine. Although there are reports in the literature of PPI-deficits induced by clozapine (Dirks et al., 2003); and present study], this enhancing effect on PPI-deficits is difficult to explain. Clozapine has mild agonistic effects on NMDA

receptors and might therefore exert some of its beneficial treatment effects by promoting NMDA receptor function, similar to glycine-site agonists (Heresco-Levy, 2003). Since both PCP and dizocilpine are open-channel blockers of the NMDA receptor–thus requiring an agonist-activated receptor for their antagonistic effects–we postulate that, in PCP- and dizocilpine-pretreated rats, the NMDA receptors are changed in such a way that clozapine has particularly strong agonistic effects on the receptor, thereby enhancing the effects of PCP and dizocilpine. The reduction of PPI by clozapine and enhancement of the PCP-induced deficit could also be due to its antagonistic effects on muscarinic acetylcholine receptors since acetylcholine is also known be involved in the mediation of PPI (Fendt and Koch, 1999; Jones and Shannon, 2000a,b).

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References

Andiné P, Widermark N, Axelsson R, Nyberg G, Olofsson U, Sandberg M. Characterization of MK-801-induced behavior as a putative rat model of psychosis. J Pharmacol Exp Ther 1999;290:1393–408.

Ault DT, Werling LL. Phencyclidine and dizocilpine modulate dopamine release from rat nucleus accumbens via sigma receptors. Eur J Pharmacol 1999;386:145–53.

Bakshi VP, Geyer MA. Multiple limbic regions mediate the disruption of prepulse inhibition produced in rats by the noncompetitive NMDA antagonist dizocilpine. J Neurosci 1998;18:8394–401.

Bast T, Zhang W-N, Feldon J, White IM. Effects of MK-801 and neuroleptics on prepulse inhibition: re-examination in two strains of rats. Pharmacol Biochem Behav 2000;67:647–58.

Braff D, Stone C, Callaway E, Geyer MA, Glick I, Bali L. Prestimulus effects on human startle reflex in normals and schizophrenics. Psychophysiology 1978;15;339–43.

Braff DL, Geyer MA, Swerdlow NR. Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. Psychopharmacology 2001;156:234–58.

Cadenhead KS, Swerdlow NR, Shafer KM, Diaz M, Braff DL. Modulation of the startle response and startle laterality in relatives of schizophrenic patients and in subjects with schizotypal personality disorder: evidence of inhibitory deficits. Am J Psychiatry 2000;157:1660–8.

Carter AJ. Antagonists of the NMDA receptor–channel complex and motor coordination. Life Sci 1995;57:917–29.

Davis M, Hitchcock JM, Rosen JB. Neural mechanisms of fear conditioning measured with the acoustic startle reflex. In: Madden JI, editor. Neurobiology of learning, emotion and affect. New York: Raven Press; 1991. p. 67–95.

Dirks A, Groenink L, Westphal KGC, Olivier JDA, Verdouw PM, van der Gugten J, et al. Reversal of startle gating deficits in transgenic mice overexpressing corticotropin-releasing factor by antipsychotic drugs. Neuropsychopharmacology 2003;28;1790–8.

Fendt M, Koch M. Cholinergic modulation of the acoustic startle response in the caudal pontine reticular nucleus of the rat. Eur J Pharmacol 1999;370:101–7.

Fendt M, Schwienbacher I, Koch M. Amygdaloid *N*-methyl-D-aspartate and γ-aminobutyric $acid_A$ receptors regulate sensorimotor gating in a dopamine-dependent way in rats. Neuroscience 2000;98:55–60.

Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR. Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. Psychopharmacology 2001;156:117–54.

Hamm AO, Weike AI, Schupp HT. The effect of neuroleptic medication on prepulse inhibition in schizophrenia patients: current status and future issues. Psychopharmacology 2001;156:259–65.

Heresco-Levy U. Glutamatergic neurotransmission modulation and the mechanisms of antipsychotic atypicality. Prog Neuro-Psychopharmacol Biol Psychiatry 2003;27:1113–23.

Hoffman HS, Ison JR. Reflex modification in the domain of startle: I. Some empirical findings and their implications for how the nervous system processes sensory input. Psychol Rev 1980;87:175–89.

Hoffman DC, Donovan H, Cassella JV. The effects of haloperidol and clozapine on the disruption of sensorimotor gating induced by the noncompetitive glutamate antagonist MK-801. Psychopharmacology 1993;111:339–44.

Iversen LL. MK-801 (dizocilpine maleate)—NMDA receptor antagonist. Neurotransmissions 1994;10:1–4.

Jentsch JD, Roth RH. The neuropsychopharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. Neuropsychopharmacology 1999;20:201–25.

Jentsch JD, Redmond Jr DE, Elsworth JD, Taylor JR, Youngren KD, Roth RH. Enduring cognitive deficits and cortical dopamine dysfunction in monkeys after long-term administration of phencyclidine. Science 1997;277:953–5.

Jentsch JD, Dazzi L, Chhatwal JP, Verrico CD, Roth RH. Reduced prefrontal cortical dopamine, but not acetylcholine, release after repeated, intermittent phencyclidine administration to rats. Neurosci Lett 1998;258:175–8.

Jones CK, Shannon HE. Effects of scopolamine in comparison with apomorphine and phencyclidine on prepulse inhibition in rats. Eur J Pharmacol 2000;391:105–12.

Jones CK, Shannon HE. Muscarinic cholinergic modulation of prepulse inhibition of the acoustic startle reflex. J Pharmacol Exp Ther 2000;294:1017–23.

Kehne JH, Gallager DW, Davis M. Strychnine: brainstem and spinal mediation of excitatory effects on acoustic startle. Eur J Pharmacol 1981;76:177–86.

Koch M. The neurobiology of startle. Prog Neurobiol 1999;59:107–28.

Koch M, Fendt M. Startle response modulation as a behavioral tool in neuropharmacology. Curr Neuropharmacol 2003;1:175–85.

Koch M, Friauf E. Glycine receptors in the caudal pontine reticular formation: are they important for the inhibition of the acoustic startle response? Brain Res 1995;671:63–72.

Koch M, Robbins TW. Special issue on the psychopharmacology of prepulse inhibition: basic and clinical studies. Psychopharmacology 2001;156:115–6.

Koek W, Woods JH, Winger GD. MK-801, a proposed noncompetitive antagonist of excitatory amino acid neurotransmission, produced phencyclidine-like behavioral effects in pigeons, rats and rhesus monkeys. J Pharmacol Exp Ther 1988;245:969–74.

Konradi C, Heckers S. Molecular aspects of glutamate dysregulation: implications for schizophrenia and its treatment. Pharmacol Ther 2003;97:153–79.

Lang PJ, Davis M, Öhman A. Fear and anxiety: animal models and human cognitive psychophysiology. J Affect Disord 2001;61;137–59.

Leaton RN, Supple Jr WF. Cerebellar vermis: essential for long-term-habituation of the acoustic startle response. Science 1986;232:513–5.

Lieberman JA, Sheitman BB, Kinon BJ. Neurochemical sensitization in the pathophysiology of schizophrenia: deficits and dysfunction in neuronal regulation and plasticity. Neuropsychopharmacology 1997;17:205–29.

Martinez ZA, Ellison GD, Geyer MA, Swerdlow NR. Effects of sustained phencyclidine exposure on sensorimotor gating of startle in rats. Neuropsychopharmacology 1999;21:28–39.

Martinez ZA, Oostwegel J, Geyer MA, Ellison GD, Swerdlow NR. "Early" and "late" effects of sustained haloperidol on apomorphine- and phencyclidine-induced sensorimotor gating deficits. Neuropsychopharmacology 2000;23:517–27.

Mele A, Thomas DN, Pert A. Different neural mechanisms underlie dizocilpine maleate- and dopamine agonist-induced locomotor activity. Neuroscience 1998;82:43–58.

Schulz B, Fendt M, Pedersen V, Koch M. Sensitization of prepulse inhibition deficits by repeated administration of dizocilpine. Psychopharmacology 2001;156:177–81.

Schwabe K, Koch M. Role of the medial prefrontal cortex in *N*-methyl-D-aspartate receptor antagonist induced sensorimotor gating deficit in rats. Neurosci Lett 2004;355:5–8.

Serretti A, De Ronchi D, Lorenzi C, Berardi D. New antipsychotics and schizophrenia: a review on efficacy and side effects. Curr Med Chem 2004;11:343–58.

Shelnutt SR, Gunnell M, Owens SM. Sexual dimorphism in phencyclidine in vitro metabolism and pharmacokinetics in rats. J Pharmacol Exp Ther 1999;290:1292–8.

Swerdlow NR, Caine SB, Geyer MA. Regionally selective effects of intracerebral dopamine infusion on sensorimotor gating of the startle reflex in rats. Psychopharmacology 1992;108:189–95.

Swerdlow NR, Varty GB, Geyer MA. Discrepant findings of clozapine effects on prepulse inhibition of startle: is it the route or the rat? Neuropsychopharmacology 1998;18:50–6.

Swerdlow NR, Martinez ZA, Hanlon FM, Platten A, Farid M, Auerbach P, et al. Toward understanding the biology of a complex phenotype: rat strain and substrain differences in the sensorimotor gating-disruptive effects of dopamine agonists. J Neurosci 2000;20:4325–36.

Swerdlow NR, Geyer MA, Braff DL. Neural circuit regulation of prepulse inhibition of startle in the rat: current knowledge and future challenges. Psychopharmacology 2001;156:194–215.

Swerdlow NR, Shoemaker JM, Crain S, Goins J, Onozuka K, Auerbach PP. Sensitivity to drug effects on prepulse inhibition in inbred and outbred rat strains. Pharmacol Biochem Behav 2004;77:291–302.

van den Buuse M. Deficient prepulse inhibition of acoustic startle in hooded-Wistar rats compared with Sprague–Dawley rats. Clin Exp Pharmacol Physiol 2003;30:254–61.

Varty GB, Higgins GA. Differences between three rat strains in sensitivity to prepulse inhibition of an acoustic startle response: influence of apomorphine and phencyclidine pretreatment. J Psychopharmacol 1994;8:148–56.

Wecker JR, Ison JR. Effects of motor activity on the elicitation and modification of the startle reflex in rats. Anim Learn Behav 1986;14:287–92.

Weike AI, Bauer U, Hamm AO. Effective neuroleptic medication removes prepulse inhibition deficits in schizophrenia patients. Biol Psychiatry 2000;47:61–70.

Wiley JL, Harvey SA, Balster RL, Nicholson KL. Affinity and specificity of *N*-methyl-D-aspartate channel blockers affect their ability to disrupt prepulse inhibition of acoustic startle in rats. Psychopharmacology 2003;165:378–85.

Wolf ME. The role of excitatory amino acids in behavioral sensitization to psychomotor stimulants. Prog Neurobiol 1998;54:679–720.

Yu B, Wang C, Liu J, Johnson KM, Gallagher JP. Adaptation to chronic PCP results in hyperfunctional NMDA and hypofunctional GABA(A) synaptic receptors. Neuroscience 2002;113:1–10.