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Activation of D_1, but not D_2 Receptors Potentiates Dizocilpine-Mediated Disruption of Prepulse Inhibition of the Startle

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Although substantial evidence has shown interactions between glutamatergic and dopaminergic systems play a cardinal role in the regulation of attentional processes, their involvement in informational filtering has been poorly investigated. Chiefly, little research has focused on functional correlations between the dopaminergic system and the mechanism of action of *N*-methyl-D-aspartate (NMDA) receptor antagonists on sensorimotor gating. The present study was targeted at evaluating whether the activation of D_1 and D_2 receptors is able to interact with the disruption of prepulse inhibition (PPI) of startle mediated by dizocilpine, a selective, noncompetitive NMDA receptor antagonist. We tested the effects of SKF 38393 ((±)-1-phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine-7,8-diol) (10 mg/kg, s.c.), a selective D_1 agonist, and quinpirole (0.3, 0.6 mg/kg, s.c.), a D_2 agonist, in rats, *per se* and in cotreatment with different doses of dizocilpine, ranging from 0.0015 to 0.15 mg/kg (s.c.). Subsequently, the effect of the D_1 antagonist SCH 23390 ((*R*)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine) (0.05, 0.1 mg/kg, s.c.) on PPI disruptions mediated by dizocilpine and by combination of dizocilpine and SKF 38393 was tested. Two further experiments were performed to verify whether the synergic effect of the D_1 agonist with dizocilpine was counteracted by effective doses of haloperidol (0.1, 0.5 mg/kg, i.p.) and clozapine (5, 10 mg/kg, i.p.). All experiments were carried out using standard procedures for the assessment of PPI of the acoustic startle reflex. SKF 38393, while unable to impair sensorimotor gating alone, induced PPI disruption in cotreatment with 0.05 and 0.15 mg/kg of dizocilpine, both ineffective *per se*. Furthermore, this effect was reversed by SCH 23390, but not by haloperidol or clozapine. Conversely, no synergistic effect was exhibited between quinpirole and dizocilpine, at any given dose. These findings suggest that D_1, but not D_2 receptors, enhance the disruptive effect of dizocilpine on PPI.
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INTRODUCTION

Substantial evidence has shown that dopaminergic modulation of glutamate inputs plays a pivotal role in the regulation of attentional processes (Kodama *et al*, 2002). In particular, the strong influence affected by dopamine on responses mediated by *N*-methyl-D-aspartate (NMDA) receptors (Clow and Jhamandas, 1989) potentially functions as a filtering device for the extraction of relevant information, either by modifying the signal-to-noise ratio (Cepeda *et al*, 1992) or by promoting orientation of attentional resources toward significant stimuli (Redgrave *et al*, 1999). Impairment of filtering mechanisms is conjectured to lead to sensory flooding and sensorimotor gating deficits (McGhie and Chapman, 1961; Braff and Geyer, 1990), which characterize several neuropsychiatric disorders (for a review see Braff *et al*, 2001), such as bipolar disorder (Saccuzzo and Braff, 1986; Perry *et al*, 2001), Huntington's disease (Swerdlow *et al*, 1995), obsessive–compulsive disorder (Swerdlow *et al*, 1993), Tourette's syndrome (Castellanos *et al*, 1996), and, particularly, schizophrenia (Braff *et al*, 1978). The most reliable operational paradigm for the measurement of sensorimotor gating mechanisms is considered the prepulse inhibition (PPI) of the acoustic startle reflex (ASR). Specifically, PPI is the reduction of the startle reflex that occurs when the startling stimulus is preceded by a weak, nonstartling prestimulus (Hoffman and

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Ison, 1980). Interestingly, both dopaminergic and glutamatergic systems are involved in the neural circuitry of sensorimotor gating and PPI can be disrupted by dopaminergic agonists and NMDA receptor antagonists (for a review see Geyer *et al*, 2001). Since PPI abolition produced by these compounds on rodents resembles effects present in psychotic patients, and some of these drugs, such as phencyclidine and amphetamine, are also known to induce schizophrenia-like symptoms in humans (Cohen *et al*, 1962; Bell, 1965; Angrist, 1994; Steinpresis, 1996), the above treatments may serve to model the sensorimotor gating deficits observed in psychosis.

The role of the dopaminergic system in sensorimotor gating has been thoroughly investigated, with evidence indicating D_2 receptors, rather than D_1, regulate PPI in rats (Peng *et al*, 1990; Geyer *et al*, 2001). Indeed, D_2 receptors play a key role in schizophrenia, as demonstrated by the fact that antipsychotics predominantly antagonize D_2 receptors (Seeman *et al*, 1975; Kapur and Mamo, 2003). The contribution of D_1 appears crucial for the reinforcement of effects mediated by D_2 receptors (Geyer *et al*, 2001). In fact, while D_1/D_2 agonists such as apomorphine produce dramatic PPI disruption, D_2 selective agonists, such as quinpirole, elicit only a mild PPI deficit in Sprague–Dawley rats, and no alterations in Wistar rats (Geyer *et al*, 1990).

The mechanisms through which NMDA receptor antagonists impair sensorimotor gating are far from being fully elucidated. Although the ability of dizocilpine, the prototypical NMDA receptor antagonist, to increase dopaminergic activity has been well described (Hiramatsu *et al*, 1989; Rao *et al*, 1990), many observations confirm that the mechanism through which it produces a robust disruption in PPI is unlikely to be mediated by D_2 receptors. A large body of literature shows D_2 selective antagonists are unable to reverse PPI disruption induced by NMDA receptor antagonists (Geyer *et al*, 1990; Johansson *et al*, 1994). Moreover, although several studies report atypical antipsychotics are effective against deficits caused by NMDA receptor antagonists (Geyer *et al*, 2001; Bakshi *et al*, 1994), the assumption remains debated in view of conflicting results on the ability of clozapine and other atypical antipsychotics to antagonize dizocilpine-mediated PPI disruption (Hoffman *et al*, 1993; Bast *et al*, 2000).

The role of D_1 receptors in the mechanisms accounting for NMDA receptor antagonists has been investigated. In particular, several studies have evidenced the inability of the D_1 antagonist SCH 23390 ((*R*)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine) to reverse PPI disruption mediated by dizocilpine and phencyclidine (Bakshi *et al*, 1994; Wedzony *et al*, 1994). Moreover, Ralph-Williams *et al* (2002) demonstrated that dizocilpine induces PPI deficits in D_1 and D_2 receptor mutant and knockout mice. This result induced the authors of the study to discard hypotheses concerning involvement of both receptors in the NMDA receptor antagonist-mediated impairment of sensorimotor gating. Nonetheless, manifold reports have underlined the activation of D_1 receptors as having synergistic effects with dizocilpine, in numerous behavioral tests (Goodwin *et al*, 1992; Verma and Kulkarni, 1992; Dall'Olio *et al*, 2000). In light of these premises, the present study was designed to elucidate interactions between D_1 and D_2 receptors with dizocilpine-mediated disruption of PPI. With this aim in mind, we planned the assessment of the effects on sensorimotor gating of dizocilpine in cotreatment with selective D_1 and D_2 receptor agonists.

MATERIALS AND METHODS

Animals

A total of 754 experimentally naïve, male Sprague–Dawley rats from Harlan Laboratories, Italy, weighing between 250 and 300 g, served as subjects. Consistent with the guidelines for housing and behavioral testing presented in the literature on startle measures in rodents (Geyer and Swerdlow, 1998), rats were housed four per cage in a room maintained at a temperature of $22 \pm 2^{\circ}$C and a humidity of 60%. Food and water were freely available and animals were held under an artificial 12/12-h light/dark cycle, with lights off from 0800 to 2000. In order to reduce stress during the experiment, each rat was gently handled for 5 min on each of the 7 days prior to behavioral testing. All experimental procedures were approved by the local ethics committee and carried out in strict accordance with the guidelines for experimental animals care (European Economic Community (86/609; DL 27/01/92, number 110)). Besides, all efforts were made to minimize the number of animals used, and sample sizes for every experiment were chosen accordingly, without ever compromising the soundness of statistical analysis.

Drugs

The following drugs were used: SKF 38393 ((±)-1-phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine-7,8-diol) hydrobromide, SCH 23390 hydrochloride, haloperidol, clozapine, and dizocilpine maleate. All drugs were purchased from Tocris Cookson, UK. SKF 38393 and SCH 23390 were dissolved in distilled water, while dizocilpine was dissolved in 0.9% saline. Haloperidol was dissolved in 10% acetic acid buffered with NaOH and diluted with saline. Clozapine was dissolved in a single drop of 1 N HCl and diluted with saline. The pH was adjusted to 5 using $NaHCO_3$. All drugs were weighed out as salts and administered in an injection volume of 1 ml/kg.

Apparatus

The apparatus used for detection of startle reflexes (Med Associates, St Albans, USA) consisted of four standard cages placed in sound-attenuated chambers with fan ventilation. Each cage consisted of a plexiglas cylinder of 9 cm diameter, mounted on a piezoelectric accelerometric platform connected to an analogue-digital converter. Background noise and acoustic bursts were conveyed by two separate speakers, each one properly placed so as to produce a variation of sound within 1 dB across the startle cage. Both speakers and startle cages were connected to a main PC, which detected and analyzed all chamber variables with specific software. Before each testing session, acoustic stimuli and mechanical responses were calibrated via specific devices supplied by Med Associates.

Procedure

At 3 days before the experiment, all rats went through a brief base-line startle session. Rats were exposed to a background noise of 70 dB and, after an acclimatization period of 5 min, were presented with a randomized sequence of 12 40-ms bursts of 115 dB, interposed with three trials in which a 82 dB prestimulus preceded the same pulse by 100 ms. Subsequently, treatment groups were established so that the average startle response and %PPI of each group were equivalent in all groups. On the testing day, each rat was placed in a cage for a 5 min acclimatization period with a 70 dB white noise background, which continued for the remainder of the session. Each session consisted of three consecutive sequences of trials (periods). Unlike the first and the third period, during which rats were presented with only five pulse-alone trials of 115 dB, the second period consisted of a pseudorandom sequence of 50 trials, including 12 pulse-alone trials, 30 trials of pulse preceded by 73, 76, or 82 dB prepulses (10 for each level of prepulse loudness), and eight no stimulus trials, where only the background noise was delivered. Intertrial intervals were selected randomly between 10 and 15 s.

Experiment Descriptions

The study was articulated in seven experiments. The first experiment was carried out to verify whether SKF 38393 synergizes with dizocilpine in disrupting PPI. A total of 144 rats were therefore pretreated with either saline or SKF 38393 (10 mg/kg, s.c), and, 5 min post-treatment, they were given injections of saline or a dose of dizocilpine (0.0015, 0.005, 0.015, 0.05, and 0.15 mg/kg, s.c.) At 5 min after this second treatment, animals were subjected to the test session. All groups consisted of 12 rats.

The second experiment ($n = 96$; 12 groups of eight rats each) evaluated the effect of quinpirole (0.3, 0.6 mg/kg, s.c.) in cotreatment with dizocilpine, at three doses (0.0015, 0.015, and 0.15 mg/kg, s.c.). Accordingly, rats were pretreated with either 0.9% saline or quinpirole (0.3, 0.6 mg/kg, s.c.). Immediately after this treatment, animals were injected either with saline or with one of the doses of dizocilpine. At 5 min after this injection, rats went through behavioral testing.

The third experiment ($n = 126$; 18 groups of seven rats each) was directed toward screening effects of SCH 23390 on PPI, both *per se* and in reversing the PPI disruption induced by the cotreatment with dizocilpine and SKF 38393, as verified in the first experiment. Therefore, rats were injected with SCH 23390, at the doses of 0.05 and 0.1 mg/kg (s.c), or saline. After 10 min, each group of rats was further injected either with saline or SKF 38393 and, 5 min later, with saline or a dose of dizocilpine (0.0015, 0.05, or 0.15 mg/kg). Finally, 20 min after SCH 23390 treatment, all rats went through startle testing.

The fourth experiment ($n = 104$) was designed to assess the ability of haloperidol (0.1, 0.5 mg/kg, i.p.) to reverse the effect induced by the cotreatment of dizocilpine and SKF 38393. The schedule of the experiment followed the same used in the third experiment, allowing a period of 60 min before startle testing for haloperidol. All groups consisted of 12 rats, with the exception of the ones that received the antipsychotic in cotreatment with dizocilpine and SKF 38393, which comprised 16 rats each.

The fifth experiment ($n = 108$, nine groups of 12 rats each) was performed to verify whether the same treatment with haloperidol used in the fourth experiment was effective on the PPI disruption induced by quinpirole (0.3, 0.6 mg/kg, s.c.), administered 5 min before startle evaluation.

The sixth experiment ($n = 104$) was aimed at verifying the effects of clozapine (5, 10 mg/kg, i.p.) on the combination with SKF 38393 and dizocilpine. The experimental procedure was identical to the one described in the third and fourth experiment, with a time interval of 40 min between the administration of clozapine and startle testing. All groups consisted of 12 rats, with the exception of the ones that were administered clozapine together with the combination of dizocilpine and SKF 38393, which comprised 16 rats each.

In the seventh experiment, we verified the effects of the same doses of clozapine used in the sixth experiment against a treatment with only dizocilpine (0.15 mg/kg, s.c.). Therefore, 72 rats (six groups of 12 rats each) were administered with either saline or one of the doses of clozapine (5, 10 mg/kg, i.p.) and, after 35 min, were treated with saline or dizocilpine (0.15 mg/kg), in order to be tested 5 min later.

Data Analysis

For each animal, the mean startle amplitudes for the first and the second halves of the second period of the session (blocks, six pulse-alone trials each) were analyzed with a three- or four-way analysis of variance (ANOVA), with pre-pretreatment (where present), pretreatment, and treatment as between-subjects factors and blocks as repeated measures. The %PPI was calculated with the following formula: $100-((\text{mean startle amplitude for prepulse} + \text{pulse trials}/\text{mean startle amplitude for pulse-alone trials}) \times 100)$ and analyzed in multifactor ANOVAs (with specific design and comparisons noted below for each experiment) with the different combinations of injections for pretreatment and treatment as between-subjects factors and trial types as repeated measures. *Post hoc* analyses were performed using Tukey's test. Alpha was set at 0.05.

RESULTS

Throughout the study no-stimulus trials data were found negligible in comparison with other startle values; therefore, they will not be presented here.

Effects of SKF 38393 Pretreatment in Cotreatment with Dizocilpine

Tested in the experiment were the effects of dizocilpine treatment at five doses (0.0015, 0.005, 0.015, 0.05, and 0.15 mg/kg, s.c.) after saline or SKF 38393 (10 mg/kg, s.c.) pretreatment. Startle magnitudes were evaluated using a three-way ANOVA (pretreatment and treatment being between-subjects factors, and blocks being repeated measures). ANOVA exhibited a significant block effect ($F(1,132) = 25.06$, $P < 0.001$). Moreover, as shown in Table 1, neither pretreatment with SKF 38393 nor treatment with

Table 1 Mean Startle Amplitudes of Treatment Groups in the First Experiment

Group	Mean SA	1st block	2nd block
sal+sal	606.64±9.45	612.22±10.67	601.06±9.86
SKF+sal	601.12±11.46	607.59±11.50	594.65±12.17
sal+diz 0.0015	606.70±13.06	618.48±14.18	594.93±12.16
sal+diz 0.005	604.94±18.09	609.20±19.46	600.68±20.70
sal+diz 0.015	605.92±8.65	611.56±8.85	600.27±8.93
sal+diz 0.05	623.56±15.24	631.29±15.04	615.84±15.57
sal+diz 0.15	628.29±14.96	635.62±14.49	620.95±15.53
SKF+diz 0.0015	601.52±13.31	607.54±13.82	595.50±12.96
SKF+diz 0.005	606.46±18.90	611.02±15.11	601.90±26.43
SKF+diz 0.015	608.26±10.39	613.35±9.84	603.17±11.48
SKF+diz 0.05	627.64±15.18	633.69±16.08	621.60±14.59
SKF+diz 0.15	623.16±14.95	629.64±14.85	616.68±15.29

Values represent mean±SEM for each treatment. All dizocilpine doses are given in mg/kg. Mean SA, mean startle amplitude for the whole trial sequence; 1st block, mean startle amplitude for the first half of the session; 2nd block, mean startle amplitude for the second half of the session; sal, saline; SKF, SKF 38393; diz, dizocilpine. Significant effects between blocks were not indicated. For further details, see text.

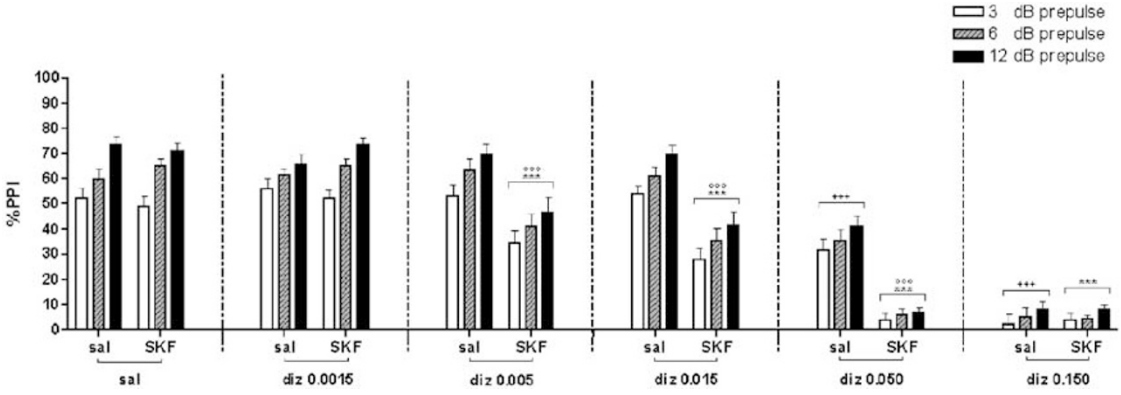

Figure 1 Effects of the synergy of dizocilpine and SKF 38393 (10 mg/kg). Pretreatments and treatment are indicated over and below the braces, respectively. All dizocilpine doses are given in mg/kg. Values represent mean±SEM. for each treatment. Prepulses are indicated by the intensity corresponding to decibels above background noise. Sal, saline; SKF, SKF 38393; diz, dizocilpine. $^{\circ\circ\circ}P<0.001$ compared with the respective sal + diz group; $^{***}P<0.001$ compared to SKF + sal group; $^{+++}P<0.001$ compared to sal + sal group.

dizocilpine significantly altered baseline startle (pretreatment: $F(1,132)=0.03$; NS; treatment: $F(5,132)=1.16$; NS, ANOVA), although the doses of 0.05 and 0.15 mg/kg dizocilpine slightly increased startle amplitude. Finally, ANOVA found no pretreatment × treatment interaction ($F(5,132)=0.05$; NS).

PPI was evaluated by a three-way, fixed factors, repeated-measures ANOVA, with pretreatment, treatment, and trial types as factors. As indicated in Figure 1, statistical evaluation remarkably attested a significant interaction between pretreatment and treatment ($F(5,132)=8.60$; $P<0.0001$, ANOVA). In detail, *post hoc* analysis proved that dizocilpine significantly disrupted PPI in comparison with saline at the doses of 0.05 and 0.15 mg/kg ($P<0.0001$ in comparison with saline), while SKF 38393 failed to elicit any alteration of PPI. Furthermore, SKF 38393 dramatically potentiated the disruptive effect of dizocilpine in the 'dose window' between 0.005 and 0.05 mg/kg at all prepulse levels ($P<0.01$ for all groups, in comparison with controls), while no such effect was observed for higher doses (presumably due to a 'floor' effect). As expected, significant differences were additionally found between different prepulse levels ($F(2,264)=171.92$; $P<0.0001$, ANOVA).

Effects of Quinpirole Pretreatment in Combination with Dizocilpine

In the second experiment, three different dizocilpine doses, ranging from 0.0015 to 0.15 mg/kg (s.c.), were administered following injection of the D_2-receptor agonist quinpirole (0.3 and 0.6 mg/kg, s.c.) or saline. A three-way ANOVA, with pretreatment and treatment as independent variables and blocks as repeated measures, was performed to scrutinize startle amplitudes. Table 2 shows that, although no fully significant effect was observed for pretreatment, a definite statistical trend was exhibited for quinpirole to increase startle amplitude ($F(2,84)=1.69$; $P<0.1$). As in the first experiment, dizocilpine weakly, but not significantly, increased ASR at the highest doses ($F(3,84)=0.47$; NS). Finally, a significant difference between block values revealed an habituation effect for startle magnitudes ($F(1,84)=12.02$; $P<0.001$).

Table 2 Mean Startle Amplitudes of Treatment Groups in the Second Experiment

Group	Mean SA	1st block	2nd block
sal+sal	610.03 ± 25.13	611.89 ± 24.29	608.18 ± 26.34
quin 0.3+sal	631.45 ± 17.09	632.97 ± 18.97	629.93 ± 15.45
quin 0.6+sal	645.00 ± 18.91	647.53 ± 20.35	642.46 ± 16.20
sal+diz 0.0015	604.35 ± 21.92	606.38 ± 22.12	602.31 ± 17.51
sal+diz 0.015	614.26 ± 21.54	616.17 ± 19.44	612.35 ± 24.02
sal+diz 0.15	639.50 ± 17.54	644.58 ± 17.63	634.43 ± 21.02
quin 0.3+diz 0.0015	635.95 ± 15.23	637.69 ± 14.43	634.21 ± 16.20
quin 0.3+diz 0.015	623.64 ± 20.56	626.21 ± 20.70	621.06 ± 20.78
quin 0.3+diz 0.15	643.26 ± 21.51	645.76 ± 21.17	640.77 ± 22.21
quin 0.6+diz 0.0015	637.37 ± 19.25	640.47 ± 17.51	634.26 ± 21.16
quin 0.6+diz 0.015	641.59 ± 13.50	645.80 ± 13.90	637.37 ± 13.53
quin 0.6+diz 0.15	643.86 ± 18.85	647.85 ± 17.25	639.87 ± 20.92

Values represent mean ± SEM for each treatment. All quinpirole and dizocilpine doses are given in mg/kg. For all groups $n = 8$. Mean SA, mean startle amplitude for the whole trial sequence; 1st block, mean startle amplitude for the first half of the session; 2nd block, mean startle amplitude for the second half of the session; sal, saline; diz, dizocilpine; quin, quinpirole. Significant effects between blocks were not indicated. For further details, see text.

A three-way ANOVA designed identically as in the previous experiment was used to analyze PPI levels. As expected, ANOVA revealed a significant effect for pretreatment ($F(2,84) = 12.30$; $P < 0.0001$, ANOVA) and *post hoc* revealed that both at the dose of 0.3 mg/kg ($P < 0.01$) or at the dose of 0.6 mg/kg ($P < 0.001$) quinpirole is able to disrupt PPI. In addition, dizocilpine was shown to disrupt PPI at the dose of 0.15 mg/kg (($F(3,84) = 93.73$; $P < 0.0001$, ANOVA); $P < 0.0005$ between 0.15 mg/kg dizocilpine and saline, Tukey) (Figure 2). Remarkably, no significant effect was detected for the interaction pretreatment × treatment ($F(6,84) = 1.58$; NS, ANOVA), showing no synergistic effect between quinpirole and dizocilpine on PPI parameters. Eventually, a significant difference between prepulse intensities was also found ($F(2,168) = 70.76$; $P < 0.0001$).

Effects of SCH Pretreatment *vs* SKF and Dizocilpine

The third experiment evaluated the capability of SCH 23390 (0.05, 0.01 mg/kg, s.c.), a D_1 receptor antagonist, in reversing the PPI disruption induced by a cotreatment with SKF (10 mg/kg, s.c.) and dizocilpine (0.015, 0.15 mg/kg, s.c.). Startle amplitudes were analyzed by a four-way ANOVA with the three series of treatments as independent variables and blocks as repeated measures. ANOVA showed neither SCH ($F(2,110) = 0.04$; NS) nor SKF ($F(1,110) = 0.01$; NS) were able to significantly alter startle amplitudes. Besides, startle magnitude increase induced by dizocilpine, as shown in Table 3, did not appear statistically significant ($F(2,110) = 3.03$; NS). Instead, ANOVA showed a significant effect for blocks ($F(1,110) = 12.12$; $P < 0.001$]. A further four-way ANOVA, with the same independent variables and with PPI levels as repeated measures, served to test PPI values. ANOVA revealed either in SCH groups ($F(2,110) = 10.92$; $P < 0.0001$) or in SKF groups ($F(1,110) = 6.44$; $P < 0.05$] a significant alteration of PPI. As expected, dizocilpine significantly reduced PPI ($F(2,110) = 289.49$; $P < 0.0001$). A significant difference between prepulse levels was also found ($F(2,220) = 280.93$; $P < 0.0001$). As in the first experiment, a significant PPI reduction was observed for the groups treated with SKF 38393 and dizocilpine in comparison with controls treated with saline and the D_1 agonist (Figure 3). Moreover, SCH 23390, at both doses antagonized the disruption induced by the cotreatment with SKF 38393 and 0.015 mg/kg dizocilpine. As expected, no antagonism of SCH 23390 was observed against the cotreatment with SKF 38393 and the dose of dizocilpine effective *per se*. Remarkably, Tukey's test revealed a statistical trend for the dose of 0.1 mg/kg SCH 23390 to attenuate the disruptive effect of 0.15 mg/kg dizocilpine + saline ($P < 0.1$).

Effects of Haloperidol Pretreatment *vs* SKF and Dizocilpine

In the fourth experiment haloperidol was intraperitoneally administered at two different doses (0.1 and 0.5 mg/kg) prior to cotreatment with SKF (10 mg/kg, s.c.) and 0.015 mg/kg (s.c.) dizocilpine. Startle amplitudes were evaluated with three-way ANOVA using the design of the previous experiments. ANOVA showed haloperidol able to significantly reduce startle magnitudes in comparison with saline ($F(2,74) = 17.98$; $P < 0.001$, ANOVA) and *post hoc* analysis assessed a significant effect both for the dose of 0.1 mg/kg ($P < 0.05$) and for the dose of 0.5 mg/kg ($P < 0.001$) of haloperidol (see Table 4). A significant effect for blocks was also found ($F(1,74) = 10.53$; $P < 0.01$, ANOVA). No effects were observed either for treatment ($F(1,74) = 0.08$; NS, ANOVA) or for interaction pretreatment × treatment ($F(2,74) = 0.00$; NS, ANOVA).

Three-way ANOVA also provided analysis of PPI values. As expected, haloperidol was not able to alter PPI ($F(2,74) = 0.48$; NS) at any given dose, while a synergistic effect between SKF and dizocilpine was confirmed ($F(1,74) = 48.12$; $P < 0.0001$, ANOVA) (see Figure 5); besides, ANOVA showed a significant effect between prepulse levels ($F(2,148) = 138.77$; $P < 0.0001$). As indicated in Figure 4, haloperidol interestingly appeared unable to

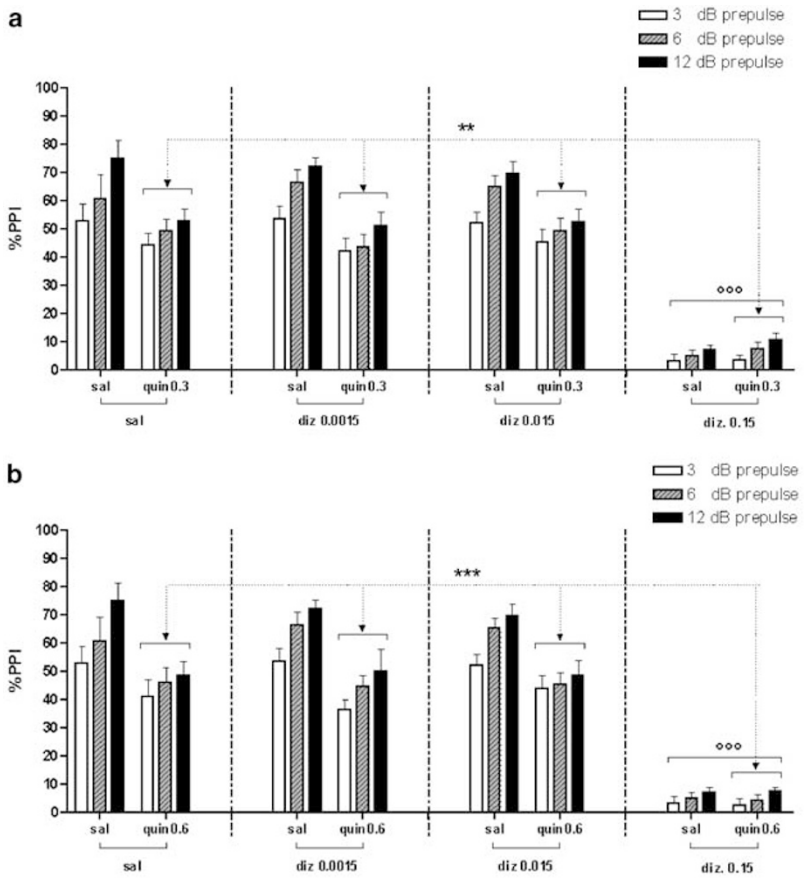

Figure 2 (a and b) Effects of the cotreatment of dizocilpine (0.0015, 0.015, and 0.15 mg/kg) and quinpirole (0.3 and 0.6 mg/kg). All quinpirole and dizocilpine doses are given in mg/kg. For all groups $n = 8$. Values represent mean ± SEM for each treatment. Prepulses are indicated by the intensity corresponding to decibels above background noise. Sal, saline; diz, dizocilpine; quin, quinpirole; $^{**}P < 0.01$, $^{***}P < 0.001$ compared to groups receiving saline pre-treatment; $^{°°°}P < 0.001$ compared to groups receiving saline treatment. For further details, see text.

reverse disruption patterns mediated by the SKF–dizocilpine cotreatment ($F(2,74) = 0.09$; NS, ANOVA).

Effects of Haloperidol Pretreatment *vs* Quinpirole

In the fifth experiment haloperidol was intraperitoneally administered at two different doses (0.1 and 0.5 mg/kg) prior to treatment with quinpirole (0.3, 0.6 mg/kg, s.c.). Startle amplitudes were evaluated with three-way ANOVA using the design of the previous experiment. ANOVA showed a significant effect for pretreatment ($F(2,99) = 35.24$; $P < 0.0001$, ANOVA) and *post hoc* analysis highlighted a significant reduction of startle magnitudes induced both by the dose of 0.1 mg/kg ($P < 0.001$) and by the dose of 0.5 mg/kg ($P < 0.001$) of haloperidol (see Table 5). A significant effect for blocks was also found ($F(1,99) = 5.20$; $P < 0.05$, ANOVA). Instead, no effect were observed for the interaction pretreatment × treatment ($F(4,99) = 0.49$; NS). Three-way ANOVA also provided analysis of PPI values. A significant pretreatment × treatment was revealed ($F(4,99) = 2.92$; $P < 0.05$) and *post hoc* analysis showed that while haloperidol is unable to alter PPI *per se* at any given dose, it totally prevents PPI disruption induced by both doses of quinpirole (see Figure 5). Moreover, ANOVA also showed a significant effect between prepulse levels ($F(2,198) = 210.27$; $P < 0.0001$).

Effects of Clozapine Pretreatment *vs* SKF and Dizocilpine

In this experiment was tested the ability of clozapine, administered at the doses of 5 mg/kg (i.p.) and 10 mg/kg (i.p.), to reverse PPI disruption induced by cotreatment with SKF (10 mg/kg, s.c.) and dizocilpine (0.015 mg/kg, s.c.). Startle magnitudes were studied in a three-way ANOVA, which showed clozapine capable, at the dose of 10 mg/kg, of significantly reducing startle amplitudes (($F(2,74) = 36.01$; $P < 0.001$, ANOVA); $P < 0.001$, Tukey) (Table 6), while no effect was observed for treatment ($F(1,74) = 0.12$; NS) or for interaction between pretreatment and treatment ($F(2,74) = 0.32$; NS) (Table 6). Moreover, ANOVA revealed a block effect ($F(1,74) = 10.96$; $P < 0.01$). PPI values analysis revealed not only that clozapine was unable to alter PPI *per se* at any given dose ($F(2,74) = 0.97$; NS] but was also ineffective in reversing PPI disruption induced by the cotreatment with the D_1 agonist and the NMDA antagonist ($F(2,74) = 0.26$; NS, ANOVA) (Figure 6). Instead, statistical analysis confirmed a significant effect for the treatment

Table 3 Mean Startle Amplitudes of Treatment Groups in the Third Experiment

Group	Mean SA	1st block	2nd block
sal+sal+sal	601.59±26.62	605.93±27.41	597.26±26.01
sal+sal+diz 0.015	599.36±15.02	605.37±14.92	599.36±15.21
sal+sal+diz 0.15	631.03±23.09	636.51±20.79	625.55±25.76
sal+SKF+sal	612.65±22.15	615.83±21.22	609.47±23.98
sal+SKF+diz 0.015	602.52±16.35	607.26±16.46	602.52±16.44
sal+SKF+diz 0.15	633.06±23.67	637.04±21.32	629.08±26.21
SCH 0.05+sal+sal	605.88±21.15	611.51±20.09	600.26±22.88
SCH 0.05+sal+diz 0.015	610.40±18.19	614.85±16.97	605.95±20.18
SCH 0.05+sal+diz 0.15	629.91±18.18	633.99±16.79	625.83±20.32
SCH 0.05+SKF+sal	606.56±19.90	610.66±19.05	602.47±21.05
SCH 0.05+SKF+diz 0.015	606.26±15.47	611.28±15.15	606.26±16.18
SCH 0.05+SKF+diz 0.15	632.02±31.64	637.02±20.41	627.02±40.44
SCH 0.1+sal+sal	608.40±19.25	613.78±19.02	608.40±19.64
SCH 0.1+sal+diz 0.015	615.23±17.75	621.72±17.12	615.23±18.77
SCH 0.1+sal+diz 0.15	629.93±23.06	631.47±17.64	628.38±35.58
SCH 0.1+SKF+sal	605.54±15.29	610.79±16.39	605.54±14.53
SCH 0.1+SKF+diz 0.015	607.75±14.86	614.04±14.02	607.75±15.94
SCH 0.1+SKF+diz 0.15	632.70±27.12	636.75±15.24	632.70±19.40

Values represent mean±SEM for each treatment. All dizocilpine and SCH 23390 doses are given in mg/kg. Mean SA, mean startle amplitude for the whole trial sequence; 1st block, mean startle amplitude for the first half of the session; 2nd block, mean startle amplitude for the second half of the session; sal, saline; diz, dizocilpine; SCH, SCH 23390; SKF, SKF 38393. Significant effects between blocks were not indicated. For further details, see text.

($F(1,74) = 63.73$; $P < 0.0001$, ANOVA) and for prepulse levels ($F(2,148) = 376.89$; $P < 0.0001$) (Figure 6).

Effects of Clozapine Pretreatment *vs* Dizocilpine

The last experiment tested the ability of clozapine, administered at the doses of 5 mg/kg (i.p.) and 10 mg/kg (i.p.), to reverse PPI disruption induced by dizocilpine (0.15 mg/kg, s.c.). A three-way ANOVA was performed to study startle magnitudes; as in the previous experiment, clozapine is able to alter startle amplitudes ($F(2,66) = 36.22$; $P < 0.001$) and *post hoc* analysis confirmed a significant reduction of startle values both for the dose of 5 mg/kg ($P < 0.05$) or for the dose of 10 mg/kg ($P < 0.001$) of clozapine (Table 7). No effect was observed for treatment ($F(1,66) = 3.60$; NS) or for interaction between pretreatment and treatment ($F(2,66) = 0.04$; NS). Moreover, ANOVA revealed a block effect ($F(1,66) = 10.04$; $P < 0.001$]. PPI values analysis showed a significant interaction pretreatment × treatment ($F(2,66) = 8.10$; $P < 0.001$] and *post hoc* analysis, revealed that both doses of clozapine are able to partially prevent PPI-disruption induced by dizocilpine (see Figure 7). Besides, as expected, *post hoc* analysis also showed that both doses of clozapine are intrinsically unable to alter PPI. ANOVA also pointed out a significant difference between PPI levels ($F(2,132) = 172.99$; $P < 0.0001$).

DISCUSSION

Consistent with previous observations (Peng *et al*, 1990; Wan *et al*, 1996), the present study revealed that both SKF 38393 and SCH 23390 are unable *per se* to alter either PPI or startle magnitude at any prepulse intensity, suggesting that neither the activation nor the blockade of D_1 receptors modifies sensorimotor gating and startle reflex. However, SKF 38393 was able to magnify dramatically the disruptive effect of dizocilpine on sensorimotor gating. Indeed, while the minimal threshold dose of dizocilpine to trigger a disruptive effect on PPI in the presence of SKF 38393 was 0.005 mg/kg, the NMDA receptor antagonist alone exhibited a comparable effect on the same parameter when administered in a dosage 10 times higher, in accordance with previous studies (Hoffman *et al*, 1993). Although the present design lacks an isobolographic analysis, and is therefore incapable of detecting pharmacological synergism *sensu stricto* in its most conclusive form (Berenbaum, 1981, 1989), our results provide clear evidence that dizocilpine powerfully potentiates the action of the D_1 agonist. While this effect was observed at doses of dizocilpine between 0.005 and 0.05 mg/kg, no significant difference was detected at higher administrations of dizocilpine, plausibly because of a 'floor effect', as mentioned in the result section. Interestingly, the effect of the combination was prevented by pretreatment with the D_1 antagonist SCH 23390, at both given doses, indicating that the observed effect is mediated by D_1 receptors.

Taken together, these data provide new and intriguing evidence on the interactions between NMDA and D_1 receptors on sensorimotor gating, and align with other evidence with regard to the ability of D_1 agonists to enhance behavioral effects of NMDA receptor antagonists, such as locomotor activity (Goodwin *et al*, 1992; Svensson *et al*, 1992; Martin *et al*, 1994) and stereotypic responses (Verma and Kulkarni, 1992). Our finding is also consistent

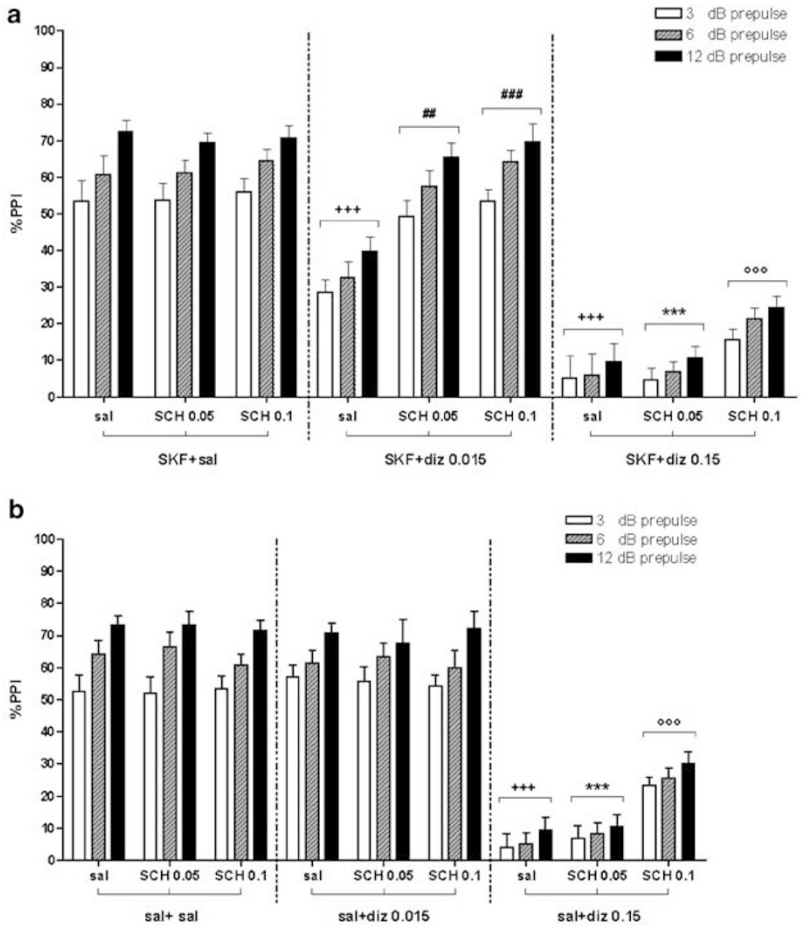

Figure 3 (a and b) Representation of the effects of SCH 23390 on the synergy SKF + diz. Prepretreatments and pretreatments + treatments are indicated over and below the braces, respectively. All dizocilpine doses are given in mg/kg. For all groups $n = 7$. Values represent mean ± SEM for each treatment. Prepulses are indicated by the intensity corresponding to decibels above background noise. Sal, saline; diz, dizocilpine; SCH, SCH 23390; SKF, SKF 23390. In graph 5 (a) $^{++}P < 0.01$, $^{+++}P < 0.001$ compared to sal + SKF + sal group; $^{\#\#}P < 0.01$ compared to sal + SKF + diz 0.015; $^{***}P < 0.001$ compared to SCH 0.05 + SKF + diz 0.15; $^{\circ\circ\circ}P < 0.001$ compared to SCH 0.1 + SKF + diz 0.15. In graph (b) $^{+++}P < 0.001$ compared to sal + sal + sal group; $^{***}P < 0.001$ compared to *SCH 0.05 + sal + diz 0.15*; $^{\circ\circ\circ}P < 0.001$ compared to SCH 0.1 + sal + diz 0.15. For further details see text.

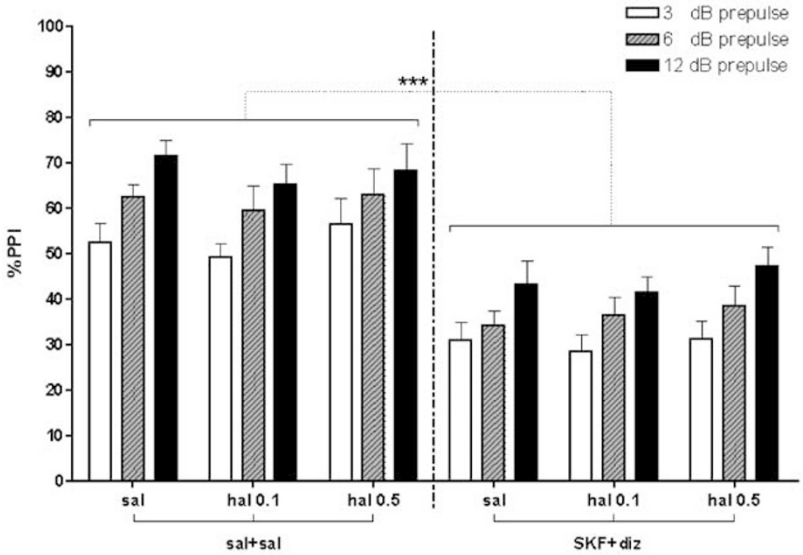

Figure 4 Representation of the effects of haloperidol on the synergy SKF + diz. Prepretreatments and pretreatments + treatments are indicated over and below the braces, respectively. All haloperidol doses are given in mg/kg. Values represent mean ± SEM for each treatment. $N = 16$ for hal 0.1 + SKF + diz and hal 0.5 + SKF + diz groups; for all remaining groups $n = 12$. Prepulses are indicated by the intensity corresponding to decibels above background noise. Sal, saline; diz, dizocilpine; hal, haloperidol; SKF, SKF 23390. $^{***}P < 0.001$ compared to sal + sal treatment groups. For further details see text.

Table 4 Mean Startle Amplitudes of Treatment Groups in the Fourth Experiment

Group	Mean SA	1st block	2nd block
sal+sal+sal	612.21 ± 17.36	616.99 ± 14.14	607.43 ± 21.43
hal 0.1+sal+sal	574.49 ± 15.67*	581.07 ± 16.00	567.90 ± 15.55
hal 0.5+sal+sal	519.17 ± 15.44***	520.75 ± 15.45	517.58 ± 15.44
sal+SKF+diz	609.77 ± 15.62	616.39 ± 15.99	603.15 ± 17.64
hal 0.1+ SKF+diz	569.24 ± 16.05*	572.74 ± 15.90	565.74 ± 16.54
hal 0.5+ SKF+diz	516.28 ± 12.18***	519.79 ± 12.41	512.78 ± 11.98

Values represent mean ± SEM for each treatment. All haloperidol doses are given in mg/kg. $N = 16$ for hal 0.1+SKF+diz and hal 0.5+SKF+diz groups; for all remaining groups $n = 12$. Mean SA, mean startle amplitude for the whole trial sequence; 1st block, mean startle amplitude for the first half of the session; 2nd block, mean startle amplitude for the second half of the session; sal, saline; hal, haloperidol; SKF, SKF 38393; diz, dizocilpine. *$P < 0.05$, ***$P < 0.001$ compared to the group pretreated with saline. Significant effects between blocks were not indicated. For further details, see text.

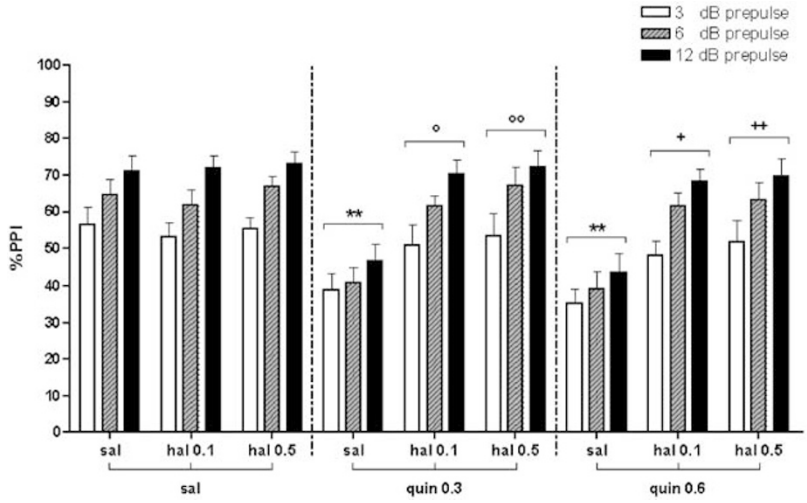

Figure 5 Effects of haloperidol pretreatment *vs* quinpirole. All haloperidol and quinpirole doses are given in mg/kg. Values represent mean ± SEM for each treatment. Prepulses are indicated by the intensity corresponding to decibels above background noise. For all groups $n = 12$. Sal, saline; hal, haloperidol; quin, quinpirole; **$P < 0.01$ in comparison with sal + sal group; °$P < 0.05$, °°$P < 0.01$ in comparison with sal + quin 0.3 group; $^{+}P < 0.05$, $^{++}P < 0.01$ in comparison with sal + quin 0.6 group. For further details, see text.

Table 5 Mean Startle Amplitudes of Treatment Groups in the Fifth Experiment

Group	Mean SA	1st block	2nd block
sal+sal	608.64 ± 15.39	612.92 ± 16.75	604.36 ± 15.74
hal 0.1+sal	576.31 ± 16.17***	580.30 ± 16.45	572.32 ± 15.92
hal 0.5+sal	528.54 ± 12.72***	534.78 ± 11.42	522.30 ± 14.46
sal+quin 0.3	636.01 ± 15.74	646.41 ± 18.64	637.11 ± 17.12
sal+quin 0.6	642.85 ± 15.34	652.90 ± 13.69	646.48 ± 23.69
hal 0.1+quin 0.3	577.27 ± 18.09***	581.82 ± 18.74	572.72 ± 25.04
hal 0.1+quin 0.6	584.01 ± 14.06***	590.07 ± 13.22	577.95 ± 15.52
hal 0.5+quin 0.3	529.31 ± 18.80***	535.37 ± 18.45	523.24 ± 19.43
hal 0.5+quin 0.6	534.72 ± 12.85***	538.27 ± 13.20	531.17 ± 13.27

Values represent mean ± SEM for each treatment. All haloperidol and quinpirole doses are given in mg/kg. For all groups $n = 12$. Mean SA, mean startle amplitude for the whole trial sequence; 1st block, mean startle amplitude for the first half of the session; 2nd block, mean startle amplitude for the second half of the session; sal, saline; hal, haloperidol; quin, quinpirole. ***$P < 0.001$ compared to the group pretreated with saline. Significant effects between blocks were not indicated. For further details, see text.

with the observation of Dall'Olio *et al* (2000) that the administration of dizocilpine prolonged SKF 38393-induced grooming in rats. Interactions between dopaminergic system and NMDA receptors appear highly complex and variable, according to the different brain regions, the type and topography of receptors, and the role of other

Table 6 Mean Startle Amplitudes of Treatment Groups in the Sixth Experiment

Group	Mean SA	1st block	2nd block
sal+sal+sal	606.29 ± 11.44	615.35 ± 12.92	597.22 ± 10.75
clo5+sal+sa	573.96 ± 19.71	576.47 ± 19.43	571.45 ± 20.92
clo10+sal+sal	486.79 ± 6.65***	486.07 ± 14.15	478.00 ± 13.76
sal+SKF+diz	604.33 ± 16.51	608.18 ± 18.08	600.48 ± 15.34
clo5+SKF+diz	571.03 ± 12.99	579.93 ± 14.00	562.12 ± 12.85
clo10+SKF+diz	498.62 ± 8.69***	524.64 ± 12.38	472.61 ± 17.18

Values represent mean ± SEM for each treatment. All clozapine doses are given in mg/kg. $N = 16$ for clo 5+SKF+diz and clo 10+SKF+diz groups; for all remaining groups $n = 12$. Mean SA, mean startle amplitude for the whole trial sequence; 1st block, mean startle amplitude for the first half of the session; 2nd block, mean startle amplitude for the second half of the session; sal, saline; clo, clozapine; SKF, SKF 38393; diz, dizocilpine. ***$P < 0.001$ compared to the groups pretreated with saline. Significant effects between blocks were not indicated. For further details, see text.

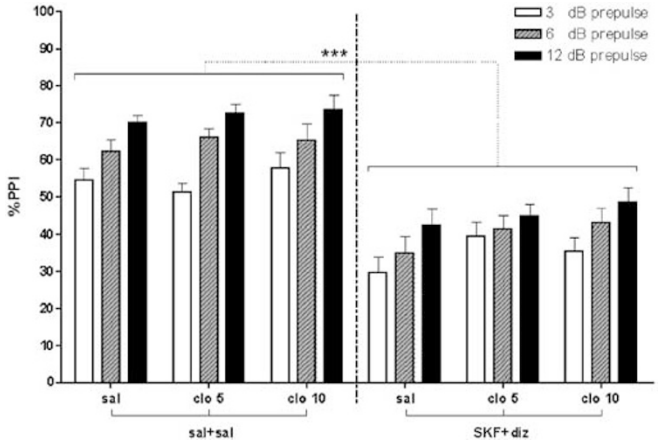

Figure 6 Representation of the effects of clozapine on the synergy SKF + diz. Pre-pretreatments and pretreatments + treatments are indicated over and below the braces, respectively. All clozapine and dizocilpine doses are given in mg/kg. Values represent mean ± SEM for each treatment. $N = 16$ for clo 5 + SKF + diz and clol 10 + SKF + diz groups; for all remaining groups $n = 12$. Prepulses are indicated by the intensity corresponding to decibels above background noise. Sal, saline; clo, clozapine; SKF, SKF 38393; diz, dizocilpine. ***$P < 0.001$ compared to sal + sal treatment groups. For further details see text.

neurotransmitters (Konradi *et al*, 2002). The body of evidence, however, indicates that one of the main areas where convergence between glutamatergic and dopaminergic fibers plays a critical role in sensorimotor gating is the core of the nucleus accumbens (NAcc) (Wan and Swerdlow, 1996). In this respect, it is worth noting that, while dizocilpine is unable to suppress PPI if infused into the NAcc, it can do so if injected into the areas that project the main glutamate inputs to this brain region, namely the lateral amygdala, the prefrontal cortex, and the dorsal hippocampus (Phillipson and Griffiths, 1985; Bakshi and Geyer, 1998). Evidence of NMDA and dopamine receptor interactions in NAcc is supported by several experimental and anatomical data (Sesack and Pickel, 1990, 1992; Youngren *et al*, 1993; Taber *et al*, 1996). On the other hand, the direction of the dopaminergic modulation of NAcc medium spiny neuron firing is known to depend on the glutamatergic tone by descending glutamate projections (Gonon and Sundstrom, 1996). Several biochemical studies indicate that low doses of NMDA receptor antagonists increase the release of glutamate (Liu and Moghaddam, 1995; Moghaddam *et al*, 1997), in all probability by preventing glutamate from driving GABAergic inhibitory neurons (Farber, 2003). It has been suggested that this increase may activate glutamatergic neurotransmission at non-NMDA receptors, like AMPA/kainate receptors (Hauber and Andersen, 1993; Olney and Farber, 1995; Bubser *et al*, 1995; Moghaddam *et al*, 1997; Deutsch *et al*, 2002). Following this evidence, a possible interpretation of our data may be based on a synergic activity of D_1 receptors on the outcomes of a general activation of NAcc mediated by non-NMDA receptors. This interpretation is in line with findings the other groups, who demonstrated that simultaneous activation of D_1 receptors and blockage of glutamate reuptake or activation of AMPA receptors in the NAcc enhances locomotor activity (Pierce *et al*, 1996; Kim *et al*, 2001). Alternatively, other areas, where dopamine is known to play a key modulatory role on glutamatergic fibers, such as prefrontal cortex, may be responsible for the effects observed in the present study. Our results, while not allowing to substantiate clearly the mechanism of action of the interactions between D_1 and NMDA receptors in the circuit subserving sensorimotor gating, stimulate further biochemical and functional investigations in this direction, to better delineate the function of D_1 receptors in the NMDA hypofunctional state.

In the third experiment, while 0.05 mg/kg SCH 23390 proved unable to prevent dizocilpine-mediated PPI disruption, the same compound significantly blunted the same effect at the dose of 0.1 mg/kg. In apparent contrast with these last results, Wedzony *et al* (1994) found that 0.1 mg/kg SCH 23390 failed to antagonize the PPI disruption mediated by dizocilpine. Nonetheless, it should be noted that the doses of NMDA receptor antagonist used in that study were higher than in our experiment. Furthermore, since SCH 23390 is known to antagonize serotonin receptors (Hicks *et al*, 1984; Briggs *et al*, 1991; Bischoff *et al*, 1986; Millan *et al*, 2001) and dizocilpine is further known to potentiate serotonergic functions (Dall'Olio *et al*, 1999), we assume that the observed intrinsic effect of SCH 23390 might depend on the blockade of serotonergic system. In fact, the role of the latter in the modulation of dizocilpine-mediated PPI abolition has been investigated by several researchers, providing controversial results. While Varty and Higgins

Table 7 Mean Startle Amplitudes of Treatment Groups in the Last Experiment

Group	Mean SA	1st block	2nd block
sal+sal	613.67±19.28	615.52±18.89	611.83±19.86
clo 5+sal	569.94±15.01*	573.63±13.64	566.25±16.53
clo 10+sal	488.18±9.87***	490.93±8.61	485.43±11.41
sal+diz 0.15	641.69±20.66	644.07±21.27	639.31±20.15
clo 5+diz 0.15	593.83±13.44*	596.58±14.24	591.08±12.69
clo 10+diz 0.15	508.02±11.36***	510.24±11.30	505.81±12.06

Values represent mean±SEM for each treatment. All clozapine doses are given in mg/kg. For all groups $n=12$. Mean SA, mean startle amplitude for the whole trial sequence; 1st block, mean startle amplitude for the first half of the session; 2nd block, mean startle amplitude for the second half of the session; sal, saline; clo, clozapine; diz, dizocilpine. *$P<0.05$, ***$P<0.001$ compared to the groups pretreated with saline. Significant effects between blocks were not indicated. For further details, see text.

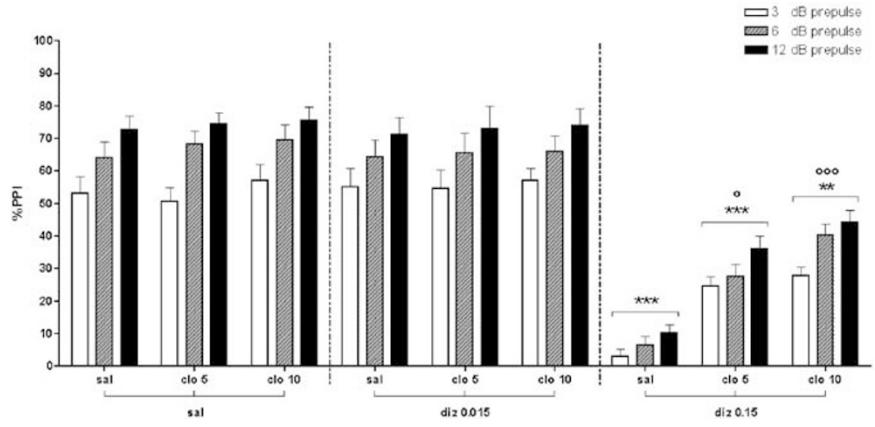

Figure 7 Effects of clozapine pretreatment *vs* dizocilpine. All clozapine doses are given in mg/kg. Values represent mean±SEM for each treatment. Prepulses are indicated by the intensity corresponding to decibels above background noise. For all groups $n=12$. Sal, saline; clo, clozapine; diz, dizocilpine. ***$P<0.001$ compared to respective control groups; °°$P<0.01$, °°°$P<0.001$ compared to sal + diz group. For further details see text.

(1995) reported that both metergoline, a $5HT_{1/2}$ antagonist, and ketanserin, a $5HT_2$ antagonist, are able to attenuate the effects of dizocilpine in PPI, Zhang *et al* (1997) found no actions of serotonergic antagonists on the same paradigm. The evaluation of this issue was beyond the aims of the present study, but the observation of this phenomenon fosters further investigation on the role of serotonin on the synergy of D_1 agonists and dizocilpine. In view of this, it is of interest that Dall'Olio *et al* (2000) demonstrated that the blockade of serotonergic system hampers the dizocilpine-induced potentiation of responses elicited by the stimulation of D_1 receptors.

No interaction between dizocilpine and D_2 receptor activation was found, in accordance with previous observations in PPI (Keith *et al*, 1991) and with other behavioral studies (Ferré *et al*, 1994; Dall'Olio *et al*, 1996), where D_2 agonists elicited either no potentiation or even counteracted some of the effects of NMDA receptor antagonists. Furthermore, haloperidol was shown unable to reverse the PPI disruption induced by the cotreatment with SKF 38393 and dizocilpine at the same doses, which were effective in preventing quinpirole-mediated PPI attenuation. Although the lack of effect of either quinpirole or haloperidol in our study might be attributable to the choice of the doses or of the route of administration, our results likely indicate that D_2 receptors are not involved in the mechanism of potentiation between D_1 receptors and the blockade of NMDA receptors.

Remarkably, dizocilpine did not significantly alter startle amplitude at any given dose either *per se* or in synergy with the D_1 agonist, although in both cases it increased startle amplitude when administered at 0.05 and 0.15 mg/kg. The evidence with regard to the effects of dizocilpine on ASR appears contradictory in the literature: in fact, while Mansbach and Geyer (1989) showed moderate (0.01–0.3 mg/kg), but not high doses (0.5–1 mg/kg) caused an overall increase in ASR amplitude, a study by Hoffman *et al* (1993), performed with a different protocol, showed dizocilpine (0.1 mg/kg) failed to alter the baseline startle amplitude; finally, Al-Amin and Schwarzkopf (1996) suggested a biphasic effect of the same compound on this parameter in Fischer rats, finding the dose of 0.05 mg/kg augmented the baseline startle, while higher doses reduced it. Taken together, the variations between these results show that the role of NMDA receptors in the neurobiological circuitry accounting for startle responses might be highly complex and possibly related to the strain of the animals, as well as other paradigm variables, like the loudness level of pulses and the intertrial time.

Rats treated with quinpirole showed an augmentation of startle reflex, although not fully significant, while haloperidol and clozapine induced a significant reduction of startle

reflex. In parallel, acute administrations of dizocilpine did not significantly alter startle amplitude at any given dose or *per se* in synergy with D_1 and D_2 agonists, although it produced an increase of startle amplitude when administered at 0.05 and 0.15 mg/kg. Our data on dopaminergic agents are consistent with other studies (Peng *et al*, 1990; Davis 1980; Davis and Aghajanian, 1976) and suggests involvement of D_2 receptors in the neural circuitry accounting for ASR magnitude.

Although our protocol is not designed to assess habituation specifically, significant differences between the two blocks of the stimuli sequence were found throughout the study, in agreement with previous reports about the inability of dopaminergic agonists (Davis and Aghajanian, 1976; Davis, 1980) to impair habituation processes. The fourth and sixth experiments showed PPI disruption caused by the association of dizocilpine and SKF 38393 cannot be attenuated either by haloperidol or by clozapine, at any given dose. In fact, although both antipsychotics are known to antagonize D_1 receptors, the given dose of D_1 agonist was arguably too high for any efficient competition to the D_1 site to occur. On the other hand, it is to stress the point that, provided the observed effect is the result of a magnification of the effect of dizocilpine, PPI disruption induced by the latter is not reversed by haloperidol, as mentioned in the introduction. The ability of clozapine to reverse PPI disruption provoked by dizocilpine is highly questioned in literature, since different studies have obtained contrasting results (Bakshi *et al*, 1994; Hoffman *et al*, 1993). Interestingly, in the present study we have shown that clozapine is able to attenuate, but not fully antagonize, dizocilpine-mediated PPI disruption in our experimental setting. In this respect, the abolition of sensorimotor gating mediated by the synergy of SKF 38393 and dizocilpine would appear to differ from the one induced by the NMDA receptor antagonist alone, indicating that the contemporary activation of D_1 agonist and blockade of NMDA receptor either potentiates chiefly the disruptive effects of dizocilpine, which are not under control of clozapine or involves also different, hitherto unknown mechanisms of action.

As mentioned in the introduction, D_1 receptors are synergic with D_2 receptors, even if the neurobiological links of this relationship remain unclear. Our study demonstrates D_1 receptors also serve the same function for PPI disruption mediated by dizocilpine. Further studies are required to understand whether this observation can be extended to other NMDA receptor antagonists, such as phencyclidine and ketamine. Whatever the mechanism, we assume that D_1 receptors play a key role as enhancers of the action of the main receptors involved in PPI disruption. Since the blockade of NMDA receptors is regarded as a putative model of negative symptoms in schizophrenia, further research is required to assess the clinical value of our observation in the management and treatment of psychotic patients.

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